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# THE LABORATORY PROPAGATION OF *SPIROMETRA MANSONOIDES* AS AN EXPERIMENTAL TOOL. I. COLLECTING, INCUBATION AND HATCHING OF THE EGGS

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Although parasitic helminths in general are increasingly the subject of biochemical, physiologic, and immunologic investigation, comparatively few species are employed in such studies due to the difficulty of propagating the worms in the laboratory. The only tapeworms which have received extensive experimental attention are the small cyclophyllideans *Hymenolepis nana, H. diminuta* and, to a lesser extent, *Taenia crassicollis, Echinococcus granulosus,* and *E. multilocularis.* The plerocercoids of certain pseudophyllidean cestodes (*Ligula, Schistocephalus, Dibothriocephalus latus,* and *Diphyllobothrium* sp.) have been used for similar investigations, but require access to particular lakes where naturally infected fishes occur. Cyclical propagation of these forms under artificial conditions is difficult or impossible, because of the wild hosts involved. Since pseudophyllidean cestodes are more primitive than their cyclophyllidean relatives, have a more expanded life history, and show important differences of vitamin $B_{12}$ content, it would appear that a pseudophyllidean which can easily be propagated in the laboratory should offer a fertile field for comparative studies.

A variety of forms are included in the genus *Spirometra.* Some of these require wild hosts, and most of them are so intergrading and poorly characterized that species definition is an unsolved problem (Iwata, 1933). *S. mansonoides* (Mueller 1935) is unique in having constant, well-defined characters (Mueller, 1936), and thrives in hosts that are easily maintained under artificial conditions.

In the spring of 1956, the author reactivated work on *S. mansonoides* with the primary purpose of attempting to culture the plerocercoid, and possibly other stages, in vitro. Since it required an abundant supply of all life history stages, practical methods had to be developed for the massive propagation of the worm. These methods have now been perfected and simplified to a point that could not have been anticipated.

The Syracuse focus of natural infection in domestic cats with *S. mansonoides,* originally reported by Mueller (1935), still exists, as does also enzootic infection in local water snakes (Mueller, 1938). Since incidence of natural infection in cats is low (2 or 3 percent), spargana were collected from water snakes. A collection of 7 *Natrix,* from Big Bay Creek, at the western end of Oneida Lake, taken on May 25, 1956, yielded 2 small spargana lying under the skin of a single snake. These spargana were fed to 2 worm-free cats on May 29. Both cats showed ova in the feces 12 to 13 days later. These 2 cats were the source of our present experimental

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stock. One of them was sacrificed on January 23, 1957. The other was carefully segregated to avoid accidental reinfection. It is still shedding large numbers of ova at the time of this writing, 2½ years later. At present we maintain a colony of approximately 12 infected cats.

*Care of Cats*

Our cats are from sources within the city of Syracuse, since city cats are less exposed to previous infection with large tapeworms than farm cats. Before an animal is added to the colony, it is quarantined for 2 weeks in separate quarters, during which time it is checked for parasites, and as an additional precaution given an all purpose anthelmintic. The animals are kept in individual metal cages 21 inches wide, 18 inches high, and 22 inches deep, with solid roof, sides, and back. (Such cages are made by the Norwich Wire Works, Norwich, N. Y.) The removable floor is of flattened expanded metal, galvanized, with meshes $1\frac{5}{8} \times \frac{5}{8}$ inches. The doors are of galvanized ½-inch wire mesh. Cages are supported in heavy angle-iron racks to form batteries of 6, 2 wide and 3 deep. Beneath each tier of cages is a metal shelf for the dropping pans, 1 to each cage. These are of stainless steel, $22 \times 28 \times 1\frac{1}{2}$ inches. Ordinarily these pans are filled with a layer of absorbent bedding: bagasse, cedar shavings, ground corn cobs, etc. Our animal quarters are air-conditioned, and cats are maintained at about 71° F. This temperature keeps them in good condition and minimizes shedding. Short-haired cats are preferred since they pass less hair in their feces, a matter of importance in the collection of ova.

Both males and females appear equally susceptible to infection with *S. mansonoides,* and thrive equally well in our quarters. We prefer young (6–8 months) cats, since they take more readily to a change of domicile. A certain amount of personal attention is desirable at the beginning, until the animals begin to eat properly and become reconciled to their surroundings. Cats which do not eat quickly sicken and die, usually from respiratory infections. Once adapted, there is no difficulty in maintaining them in good condition for 2 years or longer. Our cats become so accustomed to confinement, that many of them, if removed from their cages, jump back voluntarily. They are fed a variety of canned commercial cat foods, varying from pure low grade tuna fish to the cheaper and coarser mixtures of meat, or fish scrap and cereal. Diet is changed periodically to stimulate appetite, and occasionally a little raw liver or other fresh meat is given. A pan of clean water is provided in each cage.

Our cats are infected with varying numbers of spargana; but there appears to be little advantage in feeding more than 4, since above that number either all the adults are small, or competition results in a few large worms and suppression or stunting of the remainder, with no gain in egg production. If the animals are properly cared for, the worm appears to produce no noticeable injury to the host. For collection of ova, it is advisable to use a battery of from 4 to 6 infected cats, each with 3 or 4 worms, to insure the most efficient operation.

*Collection of Ova*

Water, as referred to in this and succeeding papers, means raw tap water, used without treatment of any kind, unless otherwise specified. Syracuse water is drawn from Skaneateles Lake and, having very low turbidity, is delivered either directly

into the mains or stored in reservoirs. It is not filtered. It is chlorinated at the source, and after leaving open reservoirs, to produce a residual of 18/100 p.p.m., but this is mostly dissipated in the conduits, so there is little or no detectable odor when water is drawn from the tap. The hardness varies from 80–120 p.p.m. (approximately 6 grains/gal). While these data are given as a matter of record, nothing in our experience indicates that water quality is of critical importance in any of this work.

It is essential for the success of the operation that the cats be placed on a highly digestible, low residue, concentrated, and adequate diet. Commercial canned cat foods contain much indigestible bulk, so that stools resulting therefrom are inconveniently large, and contain a heavy grit (muscle fragments, ground bone, etc.) which no amount of washing and screening will separate from the eggs. Fatty food is likewise objectionable, since fat droplets in the stools seriously interfere with concentrating ova. We have at various times tried lean beef, boiled chicken, pure tuna fish or salmon, but nothing works nearly as well as pure lean horse meat. Some of the packaged horse meat on the market is also unsuitable, since it contains ground-bone meal and charcoal, which again form fine, heavy, inseparable residues.

Several days before ova are collected, cages are scrubbed, bedding is removed from the dropping trays, and the trays are cleaned and polished. The cats are then placed on a diet of pure lean horse meat. This must be purchased by special arrangement directly from the packing company, since federal law prohibits interstate shipment of horse meat in the general market, unless "decharacterized" by addition of charcoal. The meat is pure lean, coarse chopped, free of ground bone, charcoal or other foreign matter, and must be specified and labeled "for scientific purposes." We secure our meat in bulk shipments from the Hill Packing Co. of Topeka, Kansas. It comes in 25-pound frozen blocks.

The 25-pound frozen blocks of horse meat are sawed on a band saw into 1-inch slabs. Any "freezer burn" is trimmed from the edges, and the slabs are then stored in waxed paper bags in the deep freeze. Each day a sufficient quantity is removed to the cold room (4° C) for thawing, prior to feeding the following day, care being taken to avoid waste and spoilage through over-feeding. Cats are fed for 2 days on this diet to clear the bowel, at which time cages and pans are again scrubbed. We are now ready to collect stools. Cats can be maintained on a pure horse meat diet for as long as a month without showing any serious loss of appetite, but ordinarily it is better to limit this regimen to about 2 weeks, since after that time a fall-off in egg production occurs, and sometimes large pieces of strobila are shed.

Since the eggs are susceptible to drying, the feces should be collected several times a day, the pans being scrubbed and polished after each collection. The stools resemble beef extract. They are black, tarry, small in volume, and show a tremendous concentration of ova in a finely divided matrix. Feces are collected from 4 to 6 cats simultaneously, and pooled in a beaker of water for emulsification in the cold room at 4° C. Once a day, the accumulation for the past 24 hours is processed. If the feces stand longer, even in the cold room, swelling of collagenous elements causes the mixture to develop a slimy consistency which impedes processing and results in a greatly reduced yield of eggs.

The beaker is removed from the cold room, and gently stirred with a stick or spatula to break up lumps. (Violent agitation or beating, as in a Waring Blendor, chops up hair in the stool, and again produces a residue than cannot be separated

from the eggs.) The emulsion is then poured through an ordinary kitchen wire strainer (12 meshes/inch) to remove hair. Any wad of hair is removed from the screen, loosely sloshed in water to free entangled eggs, and again screened. A small wooden potato masher may be used to work the feces through the strainer, and later on for jarring the sieves in the screening process.

The suspension is now rapidly passed through a graded set of Fisher "Mesh Series" sieves, 5 inches in diameter, by 1½ inches deep: 20, 40, 60, 100, 150, 200 meshes/inch, one at a time. The fecal suspension at this stage amounts to approximately 1 liter. The best receptacles for screening are a pair of stainless steel, seamless pans, 7 inches in diameter and 3¼ inches high. (These pans are also made by the Norwich Wire Works.) The screens are wetted beforehand. The suspension passes through quickly with little clogging of the mesh. In the finer meshes, jarring the edge of the sieve with a block of wood facilitates passage. The suspension is passed through the sieves from one pan to the other without allowing time for the eggs to settle. A little wash water in each pan, thrown onto the sieve at the end of each step, prevents loss of ova and rinses the pan for the next operation.

After the last sieving, the suspension is poured into a series of glass saucers (fig. 1) 6 inches in diameter and 1 inch in depth, with flat bottoms and evenly curved edges. (Such saucers, formerly made by the Cambridge Glass Company, Cambridge, Ohio, are, regrettably, no longer obtainable.) As many as 6 or more saucers may be required, since the suspension, due to addition of wash water, has usually swelled to 1½ liters or more. The saucers are filled almost to the brim, and allowed to stand for approximately 5 minutes. They are then serially decanted with a slow steady movement, each into a clean saucer. The eggs settle rapidly, and after contact with the bottom of the dish stick to the glass, while the lighter debris floats away. The last drop can be shaken from the saucer so that only a moist frosting of ova remains adhering to the inner surface. This frosting is visible to the naked eye, and should be checked under a dissecting binocular (3× objective, 12× ocular) to determine success of the operation. When a poor quality of horse meat is used, the eggs are badly contaminated with grit. If the dishes are permitted to stand too long, there is a viscous muddy sediment that requires resuspension and repetition of the operation.

The eggs are now washed from the dish into a beaker, with a stream of water from a small polyethylene wash bottle, and the dish used to receive the next decantation. (A number of small polyethylene "squeeze" bottles are indispensible.) By the time the last saucer has been attended to, the first is usually ready again. The process of settling and decanting is repeated 3 times, after which a point of diminishing returns sets in. When the process is properly done, eggs in enormous numbers are obtained approximately 70 percent free of foreign material.

After 3 or more decantings, the fecal suspension is discarded. The concentrated ova are stirred, poured into 1 or more saucers, as may be required, and settling and decanting repeated. At this point the eggs are almost 100 percent clean. They may be washed and sedimented in a beaker several more times before storage. Clean concentrated eggs have a grayish color when seen in bulk, somewhat like the appearance of wet sand. A brownish tinge is evidence of fecal contamination. Once cleaned, the eggs are pooled, and stored in water, in a stoppered bottle, in the cold room, at 4° C, where they keep for months without deterioration.

Eggs kept at room temperature, or in the cold room, particularly if much con-

tamination is present, are frequently attacked by microorganisms, particularly a chytrid fungus, identical with or similar to that reported by Butler and Humphries (1932) as parasitizing the ova of *Fasciola hepatica*, which if unchecked will destroy the entire culture. To prevent this, a few drops of alcoholic iodine solution may be occasionally added to the water in which the eggs are stored. Eggs are highly resistant to iodine, even in sufficient quantity to give a straw-colored tinge and definite odor to the water. It eventually disappears either by evaporation or by organic combination.

*Hatching of Ova*

All workers who have attempted the hatching of pseudophyllidean eggs have noted the extreme variation in their rate of development and time of hatching. At the beginning of this work, hatching was carried on in glass finger bowls, 8 inches in diameter. Water was changed daily. There was always considerable loss of eggs through bacterial and fungus growth, and the ova agglutinated into clumps that could not be broken up. In such cultures the rate of development is very uneven. Perhaps 10 percent of the ova develop to maturity, and hatch in 12 to 20 days, while the remainder show no change whatever, or only very retarded development. Thomas (1947) used a paddle wheel in an aquarium measuring $8 \times 12 \times 8$ inches to secure "a continual flow of water over the eggs" of *Diphyllobothrium oblongatum*, to keep them clean and produce better hatching. It is obvious that he was dealing with only small numbers of eggs, but whether he secured any degree of agitation of the eggs, or only a continuous circulation of water is not clear from his account. Smyth (1955) devised a system for the aseptic collection of *Schistocephalus* eggs and their incubation in a plastic dialysis loop. This system is unnecessarily complicated, of limited capacity, and can at best yield only very small numbers of coracidia. The best claimed for it is 77 percent normally developing eggs.

After much experimentation, the following system has been developed, which is capable of handling massive quantities of ova, assures uniform development, and yields large numbers of densely concentrated coracidia. Two or 3 ml of clean eggs, freshly collected, or from stock, are placed in a heavy-walled Ehrlenmeyer flask, 250 ml capacity, to form a layer approximately 2 mm deep. The flask is provided with a 2 holed No. 6½ rubber stopper, one hole of which is fitted with a burette-filling funnel or short-stemmed thistle funnel 4 cm in diameter, the stem of which is flush with the bottom of the stopper. A snugly fitting plastic tube, 5.5 mm in diameter, is passed through the other hole, so that a length of 8 cm hangs below the stopper. Into the end of the plastic tube is fitted a grade 2, cylindrical air stone, 25 mm high and 22 mm in diameter, provided with a metal stem. Plastic tubing and airstones of this type are obtainable in pet shops handling aquarium supplies, as are also small needle valves and manifolds, for hooking up with the compressed air line of the laboratory (fig. 2).

Several flasks may be fitted and processed simultaneously. The flasks are filled with water to a depth of approximately 5 cm, or a little above the top of the airstones, and are clamped upright on the cradle of a shaking machine (Aloe electrical universal, variable speed, Model 73030) with a ¾-inch stroke. The air valves are adjusted to give a steady diffusion of small bubbles through the flask contents, and the flasks agitated at the rate of 3 cycles (6 strokes) per second or faster. It may

be necessary to place a rheostat (Powerstat, Superior Electric Company, type 116, 0–149 V, 7½ amps) into the circuit for better regulation. Agitation and aeration are continued for 24 hours a day.

The flasks must be adjusted in such a way that the airstones do not strike the glass walls, since this results in cumulative destruction of eggs and foaming of the contents. Care must be taken that nodes do not develop in the flasks, for in such case the eggs settle out and defeat the purpose of the system. When this occurs, the situation can be remedied by changing the amount of water in the flask, or the rate of shaking. When the system is properly adjusted, the entire mass of eggs is kept in uniform suspension, no agglutination occurs, and development proceeds equally in 100 percent of the ova. Agitation in no way interferes with normal development. Not shown in drawing (fig. 2) is the small Petri dish, 45 mm in diam, with a strip of masking tape pasted inside to prevent rattling, that forms a cover for the funnel. Any eggs deposited on the sides of the funnel as a result of bubbling are thus protected from drying, and may be washed back into the flask once a day.

Incubation (with aeration and agitation) is carried on at room temperature (25°–27° C). Every 2 or 3 days the machine is stopped, the ova permitted to settle, and a sample withdrawn with a sterile Pasteur pipette for microscopic examination. If the water has become cloudy it is decanted and replaced. If contamination develops, a few drops of iodine are added. At the end of 10 days, more or less, depending upon the prevailing temperature, practically 100 percent of the eggs show active oncospheres, and some of them begin to hatch. The flasks are now removed from the shaking machine, stoppered with solid rubber stoppers, and placed in the cold room at 4° C, where they remain indefinitely without deterioration. These flasks provide a convenient and ready source of coracidia. They should be periodically checked for contamination.

When a hatch of coracidia is required, a flask is removed from storage, the water changed, and again placed on the aerating and shaking machine overnight. Progress of hatching is observed with an adaptation of standard optical equipment, as follows. A pillar binocular stereoscopic microscope (AO Spencer, series 23 LF, with inclined binocular body, triple nosepiece, with 1.0×, 2.0×, and 3.0× objectives, and 9× oculars) is adjusted with the base to the right, and the horizontal arm clamped to hold the axis of the objectives in a horizontal position. The body is then rotated so that the eyepieces incline upward (fig. 4). The eyepieces are approximately 12 inches above table level. Several inches of horizontal excursion are provided by the focusing gear, while several inches of vertical excursion are provided by the rack and pinion of the supporting pillar. A wooden or metal frame is arranged to form a shelf at the focal plane of the binocular, 9½ inches above the level of the table. A dark or black background is essential. A small spot light (AO Spencer, No. 353, Universal Microscope Illuminator, with focusing condenser system and variable transformer) is clamped on a ring stand so as to deliver a horizontal beam, at a level approximately 13 inches above the table.

The flask of eggs, having been removed from the shaking machine is filled with water to the neck, placed in strong direct sunlight for several hours, with occasional shaking, and permitted to settle. It is then placed on the support at the focus of the binocular against the dark background, and the beam from the illuminator directed through it, near the front wall, from the left (fig. 3). The resulting dark field effect

shows the coracidia as tiny scintillating ciliated spheres. If contamination has been properly controlled, no ciliates will be present to confuse the picture. Coracidia swim in straight lines and in random directions, like gas molecules, spiralling slightly around the central direction of motion. Occasionally one will execute several rapid gyrations before resuming its forward path. They exhibit complete indifference to gravity. In a freshly aerated and settled flask, they are evenly dispersed, moving in all directions. After the flask has stood for some hours, the coracidia begin to aggregate in streams, or near the top, but this is obviously a response to chemical gradients.

The failure of coracidia to orient with reference to gravity suggests that their specific gravity must be very close to 1.0. This is borne out by the impossibility of concentrating them by ordinary centrifugation. We have spun these larvae at speeds of from 20 to 30 thousand rpm (forces in the neighborhood of 22,000 g) without achieving any noticeable throwdown. For this reason, the only way to obtain dense concentrations of coracidia is the simultaneous hatching of large numbers of eggs within limited space, such as the above method permits.

Once the eggs begin to hatch in numbers, several harvests can be made in quick succession by such stimuli as change of water, change of temperature, or strong sunlight. After a hatch of coracidia has been poured off, it suffices to replenish the water, stopper the bottle, shake a few times, and set in direct sunlight again, to obtain a further hatch. After several harvests, the hatching slows down. The flask can then be given additional aeration on the shaking machine, or replaced in the cold room for storage. Another massive hatch of coracidia frequently appears shortly after return to the cold room.

A batch of eggs which has been partially hatched and stored can be induced to undergo another round of hatching by further aeration on the shaking machine. After about 10 hatches, however, few coracidia appear, and the sediment will be found to consist of empty shells, a few defective eggs with deformed contents, and very few (much less than 1 percent) eggs in the normal pre-hatching state. This is very different from lots of eggs hatched by other methods where, even after months of incubation, some eggs are in midphase, while many have not even begun to develop. The first hatches of coracidia appear to be slightly larger and swim more vigorously than the final crops.

Huge concentrated harvests of coracidia can be obtained by this method, the number and density being limited only by the number of eggs present; and very large numbers of eggs can be handled in a single flask. This is important if coracidia are to be used for immunologic investigations or for the purpose of infecting the copepod host under controlled conditions, as will be described in part II.

ACKNOWLEDGMENTS

The writer is indebted to Professor Wilford Dence, of the New York State College of Forestry for his help in collecting the infected water snakes which formed the starting point of the present study. My thanks go to Miss Marjorie Smith, and Mr. Robert Buck for assistance in the care of animals and in other routine phases of the work. Miss Winifred Osborne and Dr. Alvin Marucci have verified the resistance of coracidia to centrifugation, and have been helpful in other phases of the problem. Finally, I am indebted to Mr. Nicholas Apgar for drawing the figures.

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SUMMARY

1. Simple methods have been worked out for the massive laboratory propagation of *Spirometra mansonoides*, thus providing a pseudophyllidean cestode, any stage of which is available for experimental investigations.

2. Directions are given for the maintenance of a colony of infected cats. These are fed a diet of pure horse meat when feces are to be processed for eggs.

3. Eggs are collected practically 100 percent free of foreign matter by a rapid sieving and decanting process. They can be stored indefinitely in the cold room at 4° C in any stage of development.

4. Contamination of cultures is controlled by adding iodine to the water.

5. Using forced aeration and agitation on a shaking machine during the entire period of incubation (approximately 10 days at 25–27° C), uniform development of eggs is secured, and simultaneous hatching of massive numbers of coracidia can be produced at will.

6. Dense concentrations of coracidia for experimental purposes can be obtained by this technique.

7. Coracidia are resistant to centrifugation at forces close to 22,000 g. This is interpreted as evidence that their specific gravity is close to 1.0.

8. Direct sunlight, change of water or agitation, rise or fall of temperature, all appear to stimulate hatching in ripe eggs. But the most effective trigger seems to be bright direct sunlight.

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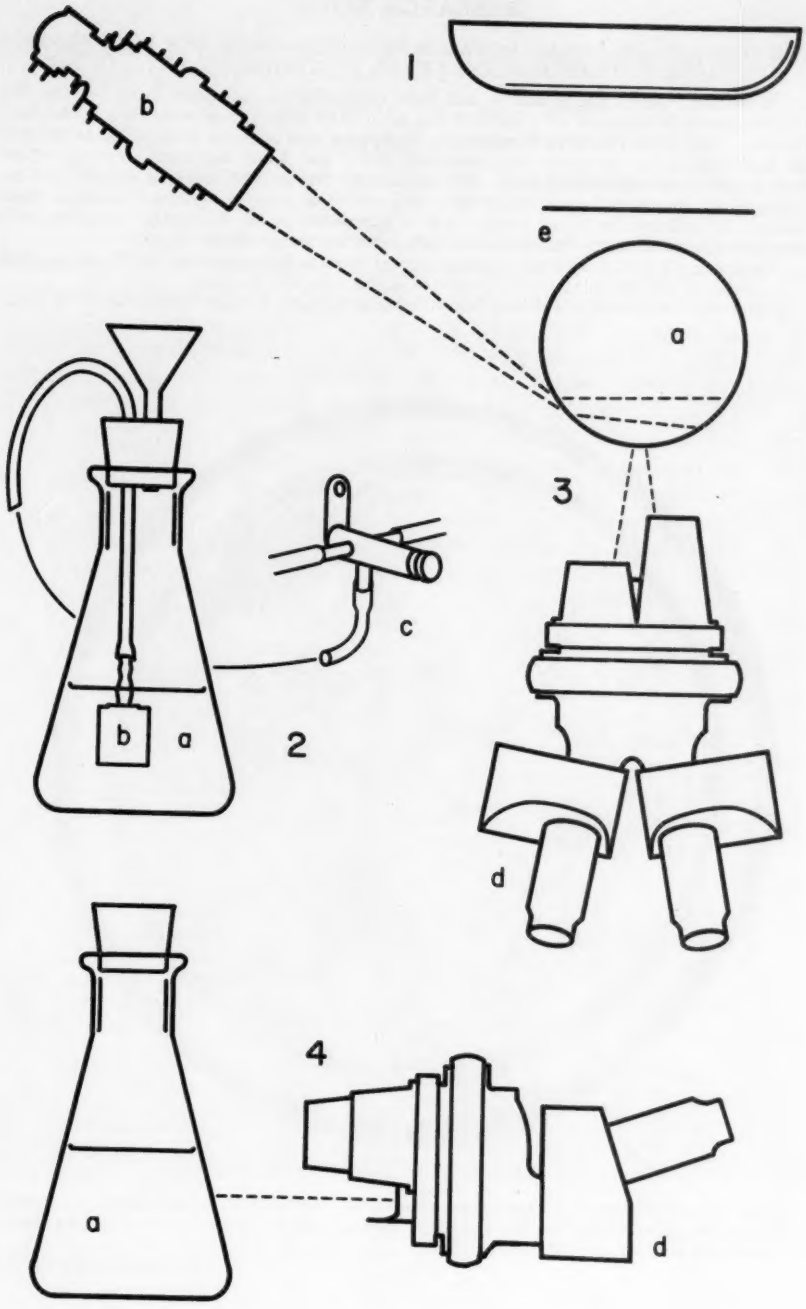
## Explanation of Plate

Figure 1. Contour of 6-inch glass saucers used in collecting and cleaning ova.

Figure 2. Arrangement of flask used for incubation and hatching ova. (The flask is clamped on the cradle of a shaking machine, and agitated continuously at 3 cycles/second, for 10 days. (a) suspension of ova; (b) grade 2 air stone; (c) air line and control valve.)

Figures 3 and 4. Top and side views of arrangement for darkfield observation of coracidia, to check hatching. (a) flask of hatching ova; (b) focusing spotlight; (d) binocular body, objective axis and focusing rack and pinion in horizontal plane; (e) black velvet background.

PLATE I



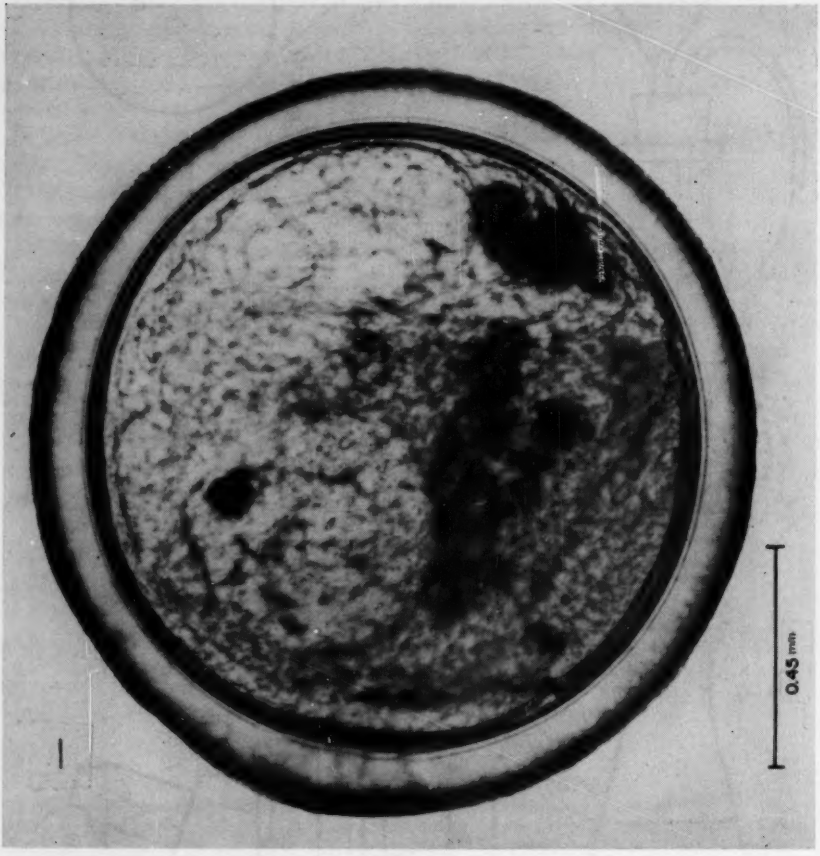
## RESEARCH NOTE

### THE IDENTITY OF METACERCARIA B REPORTED FROM THE PINK SHRIMP, *PENAEUS DUORARUM* BURKENROAD, BY WOODBURN ET AL IN 1957

Woodburn, Eldred, Clark, Hutton, and Ingle (1957, Fla. St. Bd. Cons. Tech. Ser. No. **21**: 1–33) reported the presence of a metacercaria in juvenile pink shrimp from Boca Ciega Bay, Florida. They called this larva Metacercaria B, reported that an exact count of the larvae was not made though the infections were extremely heavy, and found the worms encysted in the flesh, concentrated around many of the internal organs. Our later observations indicate that the infections are not always heavy, sometimes so few as two or three cysts being found in a single shrimp. In addition, the larvae appear to have a predilection for the ventral cephalothoracic and abdominal musculature, the latter seemingly being the preferred site.

One of us (R.F.H.) reported the metacercarial cysts in the publication by Woodburn et al and ascertained that the metacercariae reported in this note are the same.

In connection with studies of shrimp migration using parasites as tags, cysts (Fig. 1) of Meta-




cercaria B were dissected apart with fine needles and identified as an undetermined species of *Microphallus* Ward, 1901 (Fig. 2). To our knowledge, this is the first report of a *Microphallus* metacercaria from the pink shrimp.

(*continued on p. 378*)

# LIFE HISTORY, MORPHOLOGY AND TAXONOMY OF *ATRIOTAENIA* (*ERSHOVIA*) *PROCYONIS* (CESTODA: LINSTOWIIDAE), A PARASITE OF THE RACCOON*

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While investigating the intestinal parasites of vertebrates from Franklin County, Ohio, a high level of tapeworm infection was encountered in the raccoon (*Procyon lotor lotor*). This tapeworm proved to be one of the linstowids originally described by Chandler (1942) as *Oochoristica procyonis*. Since these specimens seemed to disagree in some respects with the species description, an extensive study of the morphology was undertaken in order to define more completely the species limits. The clarification of the characteristics of this cestode brought up the problem of its proper taxonomic position. As a result of investigation into this problem, the author decided that this species should be referred to as *Atriotaenia* (*Ershovia*) *procyonis* (Chandler, 1942) Spassky, 1951. The reasons are presented in the discussion. Further investigation revealed that the life history was not known. With ample material readily available, the life history of this cestode was completed in the laboratory. Extensive trapping activities led to a survey of the incidence in feral raccoons.

## MATERIALS AND METHODS

Cestodes were obtained by trapping raccoons with especially constructed live traps. Trapped animals were housed in outdoor pens and served as a source of gravid proglottids and eggs used in feeding experiments. Five suckling, 2-week-old raccoons were obtained for future use in life history experiments. These were subsequently weaned and maintained on a diet of fresh ground beef, dog chow checkers, and water. These animals were kept in indoor cages, well isolated from the adult, infected raccoons. Fecal examinations were made periodically to determine whether the animals were still uninfected. They all remained so until used for feeding experiments. Cultures of the flour beetle *Tribolium castaneum* (Hbst.), used as an experimental intermediate host, were maintained in large mason jars and fed on a mixture of wheat flour and brewers yeast. These cultures were kept at room temperatures which varied from 22° to 29° C.

Gravid proglottids used in feeding experiments were obtained either from the feces of infected raccoons or from living worms recovered from sacrificed animals. Two proglottids were placed on a small piece of filter paper, moistened, and teased apart with two small needles. In each feeding experiment, 10 beetles, starved for the previous 48 hours, were allowed to feed on the proglottids for 24 hours. These beetles were then returned to culture jars and left undisturbed until they were examined for stages of cysticercoid development. When dissecting beetles, the body (after removal of head, elytra, wings and legs) was placed in a drop of insect saline solution, teased apart, and the larval stages removed. During these dissections, care was taken to keep the entire digestive tract of the insect intact so that examination for early larval stages penetrating the gut could be carried out. Cysticercoids recovered from beetles, when fed to uninfected raccoons, were embedded in small pieces of ground beef about the size of the human thumb. Daily fecal examinations for gravid proglottids were then carried out. Some of the raccoons infected in this manner were sacrificed, and both immature and adult worms were removed from the small intestine.

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Living larval stages were studied and measured in either tap water or insect saline solution. There was no distortion of specimens, since cover glasses were not placed on fresh slide preparations. Some larvae were fixed in either 10 percent formalin or corrosive sublimate, and stained with either coelestin blue or Harris' hematoxylin. Slides showing cysticercoids in situ were made by fixing the body of the insect in Bouin's solution heated to 70° C. Sectioning was done at 30 microns and staining with Ehrlich's hematoxylin and eosin. Adult worms were relaxed in tap water, refrigerated, and then fixed in either Lavdovsky's AFA or corrosive sublimate. Such specimens were stained with Ehrlich's or Delafield's hematoxylin or Semichon's carmine. Onchospheres were stained with neutral red and observed under the phase microscope. Drawings were made with the aid of either a microprojector or a camera lucida. Measurements are from both living and fixed specimens.

## RESULTS

### *Occurrence in Raccoons*

From the spring of 1955 to the spring of 1957, a total of 29 raccoons (*Procyon lotor lotor*) were examined for intestinal parasites. Twenty-two, representing 75.8 percent, harbored *Atriotaenia* (*Ershovia*) *procyonis* in the small intestine. Infested animals were recovered from Franklin, Fairfield, Delaware, Ottawa, Perry and Madison counties. Recovery of these specimens established host records for these localities. Chandler (1942) reported this species from raccoons from Angelina County, Texas. This cestode has also been found in raccoons from southern Wisconsin (Schiller and Morgan, 1949) and from Alameda County, California (Voge, 1955). The latter author also reported this parasite from the striped skunk (*Mephitis mephitis*) from Butte County, California. McNeil and Crogsdale (1953) did not find *A.* (*E.*) *procyonis* in raccoons from Pacific County, Washington. Before the species was named, Leigh (1940) recovered immature specimens of this genus from raccoons from Tascola, Illinois.

### *Experimental Infection of Beetles and Raccoons*

Rentdorff (1948) and Millemann (1955), in establishing the life histories of *Mathevotaenia symmetrica* (= *Oochoristica ratti*) and *M. deserti* (= *O. deserti*), utilized various coleoptera and lepidoptera (including *Tribolium* spp.) as intermediate hosts. Millemann and Read (1953) reported that cysticercoids of *O. scelopori* and an unidentified species of *Oochoristica* (?) from the kangaroo rat develop in the confused flour beetle, *Tribolium confusum*. In view of the above results, it was decided to investigate *Tribolium castaneum* as a possible intermediate host of *Atriotaenia* (*Ershovia*) *procyonis*. When cysticercoid development was found to occur in this beetle, it was utilized in further life history studies. In order to determine whether hexacanth larvae were immediately infective, or whether aging was necessary for infection to result, eggs of varying ages (from autopsied raccoons) were fed to beetles. Eggs were aged on moistened filter paper, with additional water periodically added to prevent dehydration. Beetles were examined 15 days after feeding to determine whether or not infection had resulted. Twenty-four of 59 beetles (41 percent) fed eggs less than 24 hours old were found to harbor cysticercoids. At 40 to 44 hours of aging, only 8 percent harbored cysticercoids. All beetles were negative when fed eggs aged more than 48 hours. Further feeding experiments, therefore, utilized gravid proglottids from raccoon feces less than 24 hours old. The number of cysticercoids recovered from beetles usually varied from 1 to 20. From all appearances, the number of larvae in the beetle did not seem to have any effect on the rate of development or size of the cysticercoids.

In order to determine how long it took for cysticercoids to become infective, un-

infected raccoons were fed larval stages of varying ages. Cysticercoids that were 10 days old or older produced infection. The oldest cysticercoids that produced infection in raccoons were 114 days old. Adult cestodes produced by feeding 10-, 14-, and 32-day cysticercoids to uninfested raccoons produced gravid proglottids containing viable eggs immediately infective to beetles in 12 days. Raccoons fed 114-day-old cysticercoids did not pass gravid proglottids with infective hexacanths until 17 days after feeding.

*The Life History*

*The Egg.* Eggs recovered from gravid proglottids are fully embryonated and contain infective onchospheres. The egg shell varies in shape from oval to round, and measures 33 to 45 by 25 to 41 microns (plate I, fig. 1 and plate II, fig. 18). Eggs are distributed singly within the parenchyma of the gravid proglottid, a definite uterus not being present (plate II, figs. 17, 18). Eggs and their enclosed hexacanths are retained within the gravid proglottids long after the feces have been voided.

*The Onchosphere.* Hook movement within the onchosphere can be seen through the egg shell in the gravid proglottid. There is a slight space between the onchosphere and the egg shell enclosing it (plate I, fig. 1 and plate II, fig. 18). For orientation purposes, the anterior end of the hexacanth is considered as the one foremost in locomotion. This is the area bearing the hooks. The blades of the median pair of hooks point toward the ventral surface. Living, freshly hatched onchospheres measure 30 to 41 by 24 to 33 microns. The 3 pairs of embryonic hooks vary in size and shape, measuring from 13 to 17.5 microns (plate I, fig. 6 a–c). The median pair of hooks is the longest and most slender, and both hooks of this pair are similar in appearance (plate I, fig. 6 c). The members of both lateral pairs of hooks, however, are dimorphic. The shorter and broader hook of each pair is ventral, while the dorsal hook is somewhat longer and more slender (plate I, fig. 6 a, b). The blade of the ventro-lateral hook is more sharply curved than the blade of either the median or the dorsolateral hooks. While the embryonic hooks were observed in violent motion beneath the egg shell, they have never been seen to break through this shell. On the other hand, if the shell was mechanically torn by dissecting instruments, the embryos readily emerged.

Glandular areas within the onchosphere, of the type described by Reid (1948) and Ogren (1955, 1957), were readily visible when onchospheres were stained with neutral red and observed with the light microscope or in unstained onchospheres viewed through the phase microscope. In dorsal aspect, they consist of a granular pair of unicellular glands which extend from the anterior end to near the posterior end of the onchosphere. At the posterior end a narrow isthmus connects the 2 cells, resulting in a "U" shaped configuration (plate I, figs. 1–4). Seen in lateral aspect, the cells run posteriorly along the dorsal surface and near the posterior end turn ventrally and extend forward again, terminating just short of the middle of the body (plate I, fig. 5). Prior to their anterior termination, the cells divide into 2 lobes which appear to empty individually near the median pair of hooks (plate I, figs. 1, 3). The glands may change in outline through the appearance of constrictions anywhere along their length and subsequent granule flow into anterior or posterior enlargements; this results in a knobbed appearance (plate I, fig. 4). Other cells of the onchosphere vary considerably in size and number and are distributed

haphazardly, although usually located ventral to the glands (plate I, figs. 1, 2, 4, 5). A large colorless vesicle, also reported by Reid (1948), has been found attached to the posterior end of the onchosphere (plate I, figs. 3, 4). The origin and function of this vesicle is unknown.

Movement of the onchosphere seems to be accomplished chiefly by movement of the hooks. Muscular attachments to the hooks were seen only once, when it was observed that muscle strands run posteriorly from the guard of the hook. In locomotion, the median pair of hooks is thrust forward first, emerging from the body up to the point where the curved blade joins the straight handle. Each hook appears to be attached at this point, the guard, to the cuticle. These hooks then turn ventrally in a "gouging" motion until their position in the onchosphere is almost dorsoventral. By this time, the blades of both pairs of lateral hooks have emerged in an anterolateral direction and begin a "breast stroke" posteriorly. By the time they have completed their backward stroke they have a posterolateral position, and the median pair of hooks has returned to its original position, drawn back within the body of the embryo. As the median hooks again thrust forward, the lateral pairs return to their resting position within the body. This cycle then repeats, and the onchosphere moves with its anterior end foremost. The body outline changes only slightly during hook movements.

*Cysticercoid Development.* Hatching of the onchosphere takes place in the intestine of the beetle almost immediately after the egg is ingested. While the process of hatching was never observed, many empty shells and hatched onchospheres are present in the gut lumen 24 hours after feeding. Penetration of the gut wall has not yet occurred and some unhatched eggs are also present at this time. The initial stages of penetration have not been recovered, but at 38 hours onchospheres may be found in the body cavity of the beetle, attached to the outer wall of the mid-gut (plate I, fig. 7). Shortly thereafter, the embryos lie free in the hemocoel. Movement has not been observed at this stage, nor has the size increased. At 72 hours there is a slight increase in the number and size of the cells comprising the embryo, but no structural differentiation has occurred. The embryo has also increased in size, measuring 47 to 50 by 39 to 44 microns. Numerous unhatched eggs were still recovered from the intestinal lumen of beetles at this time. By the 4th day of infection larval cells have further increased in size and number, and the almost circular, non-motile embryo measures 81 to 89 by 72 to 80 microns. The cells still vary considerably in size, but differentiation has not yet occurred. There is a cavity in the center of the embryo, and the hooks are still arranged in 3 pairs (plate I, fig. 8). Embryo size continues to increase, and at 7 days has reached 117 to 147 by 109 to 140 microns. The cavity which first appeared at the 4th day of development is more posterior in position. Sucker primordia appear as light, oval areas at the anterior end. An embryonic cyst wall has started to form, but does not yet entirely enclose the embryo.

Rapid elongation quickly changes the embryo, after 8 days of development, into a definite egg-shaped larva measuring 190 to 230 by 156 to 183 microns (plate I, fig. 9). The embryonic cyst wall is now complete, and a posterior invagination marks the beginning of the formation of the excretory bladder. Irregularly shaped calcareous granules, few in number and widely scattered, make their appearance at this time. The sucker primordia are still faint, while individual cells of the embryo have become quite small and difficult to see. Invagination of the scolex be-

gins shortly after this, and may be completed at 9 days. This involution of the scolex results in partial obliteration of the cavity which was formed at 4 days of development. Due to this distortion, the cavity takes on a crescent shape (plate I, fig. 10). A slight movement of the embryo was first observed at this stage. Occasionally it will contract and relax, or slowly evaginate the scolex. With the scolex evaginated, the posterior cavity regains its original circular conformation. Although the suckers are quite distinct, their radial muscles are only partially formed. Calcareous granules have become more numerous and measure from 8 to 11 microns. Size of the larva has attained 184 to 265 by 170 to 218 microns.

The fully developed, infective cysticercoid stage is reached in 10 days. In living specimens, the calcareous granules, which measure from 8 to 12 microns, are so numerous they obscure much of the internal anatomy. When inactive, and with the scolex invaginated, the larva measures 216 to 276 by 184 to 212 microns. An active cysticercoid often evaginates its scolex and changes shape by alternately contracting and relaxing. This activity does not, however, result in locomotion. The hooks are still arranged in pairs at the posterior end of the cysticercoid, although not so closely associated with each other as they were in the onchosphere (plate I, fig. 11). The radial muscles of the suckers are completely formed and the suckers measure 60 to 70 by 44 to 55 microns. The excretory system consists of an excretory ring in the scolex from which 4 longitudinal ducts (2 dorsal and 2 ventral) extend throughout the rest of the body. At their origin from the excretory ring, both sets of ducts are of the same diameter and can not be distinguished from one another. As they course posteriorly, the dorsal ducts narrow in diameter, while the ventral ones increase slightly in diameter. The latter empty into the small excretory bladder at the posterior end of the cysticercoid (plate I, fig. 11). The narrowed dorsal ducts do not enter this bladder, but connect to each other, thereby forming a closed circuit.

While there is no change in the morphology of the cysticercoid after the tenth day of development, size continues to increase. Active, invaginated, 52-day-old cysticercoids measure 476 to 612 by 163 to 224 microns. Such specimens readily evaginate their scolices and alternately contract and relax. These contractions still do not result in movement along the substrate. The size of cysticercoids has decreased at 114 days to 252 to 401 by 177 to 292 microns, which is still greater than the size at 10 days. Cysticercoids that had been dissected from beetles were sometimes enclosed in a thin, sac-like membrane, possibly of host origin (plate I, fig. 12). A cluster of particles at one end of the sac resembles cells, but no nuclei were seen.

*Adult Stage.* Adult specimens were recovered from both feral and experimentally infected raccoons. While the step-by-step development from cysticercoid to adult was not followed in the experimental animals, several immature stages were recovered (plate II, figs. 13–15). The scolex of the adult tapeworm possesses 4 muscular suckers, but no rostellum. The craspedote strobila measures from 41 to 162 mm in length and attains a maximum width of 2.3 mm. It is composed of from 45 to 91 proglottids. The genital pores alternate irregularly. In the mature proglottid the genital ducts pass dorsal to the excretory vessels (plate II, fig. 16). Testes number from 48 to 73, and are located in a single dorsal layer between the excretory vessels posterior and lateral to the female reproductive system. The vas deferens is much coiled and anterior to the ovary on the poral side. The ovary is median,

roughly reniform, and multi-lobulate, located in the anterior median portion of the proglottid. The vitelline gland is also multi-lobulate and located posterior to the ovary. Between it and the ovary lies the rather indistinct Mehlis' gland. A pronounced seminal receptacle forms as a proximal enlargement of the vagina dorsal to the ovary. There is no evidence that a uterus exists. Gravid proglottids containing infective eggs are passed by the raccoon 12 days after ingestion of infective cysticercoids.

*Adult Morphology*

This cestode was found to conform in most morphological aspects to *Oochoristica procyonis* Chandler, 1942, but Chandler's figures for the size of this species and of its organs did not completely agree with the measurements made on specimens recovered during this study. The anatomy of the excretory system also differed from the published description. Therefore, a thorough comparison was made between the morphology of the present specimens and the type specimens. Mr. Allen McIntosh kindly supplied type specimens of *O. procyonis* from the U. S. National Museum Helminthological Collection.

Morphologically, the Ohio cestodes and the type specimens agreed in all details. While Chandler (1942) stated "uterus rapidly spreads out over entire segment. . . ," this organ was not seen in the Ohio specimens, nor was it observed by the present author in the type specimens. The eggs are enclosed singly within parenchymatous capsules (plate II, figs. 17, 18). It is a matter of interpretation whether these capsules should, collectively, be referred to as "uterus." The shape of the proglottids vary, as Chandler has stated, from wider than long (immature) to about square (mature), and finally to several times as long as wide (terminal gravid proglottids) (plate II, figs. 16, 17).

The size ranges of various organs published by Chandler are generally smaller than the size limits obtained during the present study. The maximum size attained by most organs is greater in the Ohio specimens, although size ranges from both sources overlap in most cases. The only major disagreements are in strobila length and seminal receptacle size (both measurements are greater in the present study). The size of the onchosphere hook was established by Chandler as 13 microns, while a range in hook size, as well as morphological variations, have been reported here. The maximum number of testes, originally reported as 63, has been extended to 73 in the present study. The discrepancy in sizes of structures between the original figures and those reported here is probably due to two factors: First, the type specimens appear to have contracted during fixation, while the cestodes used in the present study were fixed in a well relaxed state; Second, it seems probable that Chandler's measurements were made on but a few specimens (since he found cestodes in only 1 of 13 raccoons). Measurements reported in this paper were made on specimens from several raccoons, and 30 measurements of each structure were made. In light of the above observations, the present author feels that the cestodes encountered during this study belong to the species originally described by Chandler as *Oochoristica procyonis.*

A careful examination of the excretory system of the type specimens revealed that its true structure was overlooked in the original description. This system, in the type specimens, conforms in all respects with that of the specimens recovered in the present study. The excretory ring in the scolex is formed by the union of the

dorsal and ventral longitudinal excretory vessels and a transverse connective from each that unites the canals of the right side with those of the left side (plate III, fig. 20). These canals all have the same diameter at this point, and the ring is located between and at the anterior margins of the suckers (plate III, fig. 19). The two dorsal vessels remain unbranched and without anastomoses along their entire length. Their diameter slowly decreases, until in mature proglottids it is less than half that of the ventral vessels (plate III, fig. 22). These dorsal ducts often disappear in post-mature proglottids, and are only rarely present in gravid proglottids. Interruptions along their course end in apparently blind sacs in post-mature proglottids (plate III, fig. 23). Occasionally a small cluster of cells is grouped around the sac, the size and shape of which varies considerably.

Near the posterior margin of the suckers the 2 ventral vessels undergo abrupt and profuse branching, which results in the establishment of from 4 to 7 main longitudinal vessels along the length of the strobila (plate III, fig. 19). There are numerous anastomoses between these vessels, as well as transverse canals which connect them to each other. There is no definite pattern to the arrangement of these canals; fusion of vessels, formation of new vessels by branching, and subsequent reunion of the branches occur at random. As these vessels course posteriorly, they increase gradually in diameter, so that in mature proglottids they are at least twice the diameter of the unbranched dorsal vessels (plate III, fig. 22). The ventral excretory ducts of the terminal proglottid lead into the excretory bladder (plate III, fig. 21). After apolysis of this proglottid, the severed ends of these ducts open to the outside individually. As Chandler has stated, the ventral excretory vessels have a thinner wall than the dorsal ones.

Since the above discussion indicates some disagreement with the original species diagnosis and presents a more complete description of certain structures, it seems desirable to provide a redescription of the species at this time. The diagnosis below is based primarily on Chandler's (1942) description, with a combination of measurements presented by him with those established during this investigation. All measurements in microns, unless stated otherwise.

*Atriotaenia* (*Ershovia*) *procyonis* (Chandler, 1942) Spassky, 1951

Description: Strobila craspedote, 8 to 162 mm long, consisting of about 45 to 91 proglottids; maximum width, 2.3 mm. Scolex 270 to 430 across and 150 to 255 long, sometimes demarcated from neck by a slight constriction. Suckers 83 to 134 in diameter, in dorsal and ventral pairs; members of each pair 23 to 32 apart. Neck very short, segmentation beginning within 100 to 200 of scolex. Immature proglottids broader than long, mature proglottids about square, and terminal proglottids several times as long as broad in relaxed specimens. Mature proglottids 0.7 to 1.4 mm wide and 0.3 to 1.2 mm long; terminal ripe proglottids 0.9 to 2.3 mm wide and 1.0 to 7.2 mm long. Genital atrium 70 to 140 deep, closed by a pair of lips. Genital pores alternate irregularly, 1/5 to 1/7 length of proglottid from anterior border. Two dorsal excretory ducts narrow and upbranched, becoming more narrowed posteriorly. Ventral excretory ducts from 4 to 7 in number, with numerous cross connections and anastomoses. Ventral vessels thinner walled and about twice diameter of dorsal ones. Genital ducts pass dorsal to both sets of excretory vessels.

Testes 47 to 73 in number, round or oblong, measuring 30 to 66 by 50 to 81; situated between excretory vessels, mostly posterior and lateral to female sex glands, occasionally anterolateral or anterior, and for the most part in a single dorsal layer. Cirrus pouch 110 to 195 long by 40 to 81 wide, entering genital pouch 190 to 270 from margin of proglottid, extending far beyond excretory canals. Vas deferens much coiled anterior to ovary on poral side.

Ovary large, much branched, roughly reniform, in anterior median portion of proglottid, occupying about half its width; maximum width in mature proglottid 335 to 530. Vitelline gland also consisting of clusters of lobes, tending to become confluent in older proglottids; maximum

size 130 to 250 broad by 115 to 200 long. Vagina, opening into genital atrium from behind, nearer margin than cirrus, follows contour of cirrus for about half its length, then pursues its course to ootype in smooth curve dorsal to ovary. Seminal receptacle forms as enlargement of proximal end of vagina dorsal to ovary and not far from ootype; it measures 60 to 220 long by 30 to 85 wide in mature proglottids, but increases to 130 to 225 long by 65 to 110 wide in post mature proglottids. Mehlis' gland spherical, situated medially between ovary and vitelline gland, anterior to center of proglottid. A definite uterus not present, eggs occurring singly within parenchymatous cavities and evenly distributed throughout gravid proglottid. Ovary and vitelline glands quickly disappear, but testes, cirrus pouch, vagina and seminal receptacle persist almost to end. Onchospheres 24 to 33 by 30 to 41, with hooks 13 to 17.5 long.

Host: Raccoon, *Procyon lotor lotor.*

Location: Small intestine.

Locality: Texas, Ohio, Wisconsin, California.

## DISCUSSION

### *Taxonomic History of* Atriotaenia (Ershovia) procyonis

Tapeworms taken from Ohio raccoons were found to belong to the species originally known as *Oochoristica procyonis* Chandler, 1942. Lühe (1898) had established the genus *Oochoristica* for tapeworms of reptiles and presented such a general description that new species were added rapidly, and soon parasites of mammals were also included within the genus. In a major work on the anoplocephalid cestodes, Baer (1927) presented a more detailed description of the genus, adding several new diagnostic features. While his revision was more precise, and has been accepted by most investigators, it still contained several characteristics of questionable value. These questionable features were pointed out by Spassky (1951) in a broad revision of the anoplocephalid cestodes. He proposed a division of the genus *Oochoristica* which placed those species parasitic in mammals in 6 different genera. *O. procyonis* was placed in the genus *Atriotaenia* Sandground, 1926. (Baer's earlier revision had reduced *Atriotaenia* to synonymy with *Oochoristica.*) The genus *Atriotaenia* was established by Sandground (1926) for a tapeworm of the coati, *Nasua socialis.* His description is inadequate in some respects, while Spassky revised the genus and presented a more precise description. Spassky listed the craspedote strobila, presence of a seminal receptacle, passage of the genital ducts dorsal to the excretory ducts, and parasitism within a mammalian host as characteristics of the genus *Atriotaenia.* In the genus *Oochoristica* he included cestodes with an acraspedote strobila, without a seminal receptacle, with sex ducts passing exclusively between the excretory vessels and parasitism within a reptilian host. Prior to this reorganization, the genus *Oochoristica* had contained species which exhibited various combinations of the above mentioned characteristics, as well as some with unilateral genital pores and/or a double set of reproductive organs in each proglottid. Spassky further established 2 subgenera within the genus *Atriotaenia.* In his subgenus *Ershovia* the genital atrium is smaller than the cirrus sac and the sex ducts are dorsal to or between the excretory vessels, but dorsal to the nerve cord. Since the cestode considered in the present report fits this precise description, the author is of the opinion that it should be named *Atriotaenia* (*Ershovia*) *procyonis* (Chandler, 1942) Spassky, 1951.

Dollfus (1954) agreed with Spassky's separation of reptilian and mammalian parasites, but he favored placing all those parasitizing mammals in but one genus—*Mathevotaenia.* In a recent work, Della Santa (1956) did not accept Spassky's revision and returned the mammalian cestodes to the genus *Oochoristica.* His diagnosis of the genus is essentially that offered by Baer (1927) and Joyeux and

Baer (1936), and as such falls heir to the same objections. Species differentiation is based to a great extent upon measurements of worms and their organs, as well as ratios of one organ to another. Since these ratios are apt to vary according to the conditions of fixation, they are less reliable than anatomical differences in separating species.

At the time of its description, the genus *Atriotaenia* was placed in the family Anoplocephalidae and the subfamily Linstowinae. As previously mentioned, Baer (1927) reduced *Atriotaenia* to synonymy with *Oochoristica,* retaining the same family and subfamily designations. In 1929, Mola completely rearranged the cyclophyllidean cestodes, placing *Oochoristica* in the suborder CYCLOPHYLLANCANTHA, tribe DILEPINOIDEA and the family Dipylidiidae. This arrangement is unacceptable because descriptions of these newly established groups are sketchy and incomplete. Furthermore, *Oochoristica* was placed in a different tribe than *Linstowia,* in spite of the fact that the systematic closeness of these two genera had been adequately pointed out by Beddard (1914) and Baylis (1919). Lastly, *Oochoristica* was placed with species possessing armed scolex and rostellum; it has no such structures. Skrjabin (1933) raised the anoplocephalids to suborder rank, with the diagnosis given for the family Anoplocephalidae *sensu* Baer (1927). This placed *Oochoristica* in the suborder ANOPLOCEPHALIDATA, family Anoplocephalidae and subfamily Linstowinae. Spassky's (1951) reorganization accepted Skrjabin's suborder designation, but he further established the superfamily Anoplocephaloidea. He also recognized the family Linstowiidae (Mola, 1929) and the subfamily Linstowiinae, in which he placed the genus *Atriotaenia.* Lopez-Neyra (1954), apparently unaware of Spassky's work, retained the family Anoplocephalidae and accepted the synonymy of *Atriotaenia* with *Oochoristica.* However, he placed *Oochoristica* in the subfamily Davineinae, grouping it with genera possessing marked structural differences. His description of the genus *Oochoristica* included the presence of a uterus, which is not substantiated in the present study.

Regardless of the synonymy or independence of *Atriotaenia* and *Oochoristica,* most authors agree that they are anoplocephalids; and further, that they belong in the linstowid subgroup. The difficulty seems to be whether this classification should be considered as valid at the superfamily, family or subfamily level. The position of Baer (1927), based primarily on the classification established by Fuhrmann (1907), has been accepted by most investigators in the past. The recent works of Spassky and Lopez-Neyra are the only ones that offer extensive revisions of this position. Lopez-Neyra's systematization is unacceptable for the reasons mentioned above. The present author feels that the revision of Spassky presents the most realistic scheme of classification because of its clarity, completeness and appropriateness.

*Life History Studies*

The life history of *Atriotaenia* (*Ershovia*) *procyonis,* as completed in the laboratory, involved the beetle *Tribolium castaneum* as the intermediate host. No attempts were made to discover the natural intermediate host, though it would seem most likely to be a coprophagous beetle. All stages of development closely resemble those of other known linstowid life cycles as described by Rentdorff (1948), Millemann and Read (1953) and Millemann (1955). These workers established lepidopterous and coleopterous insects as intermediate hosts of linstowid cestodes and

were unable to infect oribatid mites (intermediate hosts in all other known anoplocephaloid life histories). While mites were not investigated as possible intermediate hosts in the present study, development in *T. castaneum* corroborates the results of the above authors, and adds weight to the recognition of the family Linstowiidae as separate and distinct from the family Anoplocephalidae.

After a suitable intermediate host ingests eggs, hatching of the onchosphere must occur before development can continue. Venard (1938) reports that in *Dipylidium caninum* the hooks do not aid in hatching. The mouth parts of the flea usually rupture the egg shell, and the embryophore is then dissolved by the digestive juices, releasing the embryo. Parodi and Alcarez (1946) reported that hatching of the onchospheres of *Multiceps serialis* and *Hymenolepis diminuta* is due to the mechanical perforation of the membranes and shell by the hooks, and not the action of the mouth parts or digestive juices of the host. Hatching of the eggs of *Atriotaenia* (*Ershovia*) *procyonis* was not observed during this study. Unhatched eggs were often recovered from the lumen of the gut of insects 72 hours after the beetles were fed. Failure of such eggs to hatch after prolonged exposure to the mechanical action of the embryonic hooks and the chemical action of the host's digestive enzymes lends credence to the probability that hatching consists of emergence of the onchosphere after the egg shell has been torn by the mouth parts of the beetle. Indeed, many empty, torn shells have been recovered from the gut lumen, indicating that some mechanical process is probably involved in hatching. It is presumed that onchospheres emerge from the torn shell by means of the movement brought about by the action of their hooks.

Morphologically, onchospheres of different species of tapeworms are difficult to distinguish from each other. Recent investigators, however, have made attempts to describe onchosphere structure more completely. As a result of this trend, glandular areas have been reported in several species (Rentdorff, 1948 and Reid, 1948). Reid's detailed description of these glands in *Raillietina cesticillus* agrees very closely with that of glands in *Atriotaenia* (*Ershovia*) *procyonis*. In *R. cesticillus*, however, these glands are anchored to the posterior end of the larva, while no such attachment occurs in *A.* (*E.*) *procyonis*. Ogren (1955, 1957) described glandular regions in *Hymenolepis nana* and *Mathevotaenia symmetrica* which closely resemble the gland cells of *A.* (*E.*) *procyonis*. The function of these glands has not been definitely determined. Reid has referred to them as penetration glands, having postulated that granule secretion aids in penetration of the intermediate host gut. Ogren (1957), however, feels that these glands might contribute to the formation of extraembryonic membranes surrounding the larva, and suggests epidermal gland as a non-functional name. The arrangement and relationships of onchosphere hooks appears to be similar in most cestode species. However, the role of embryonic hooks in onchosphere locomotion seems to vary among different species. Venard's (1938) description of onchosphere locomotion and its relationship to hook movement in *Dipylidium caninum* agrees closely with that of *A* (*E.*) *procyonis*. Parodi and Alcarez (1946), observing the onchospheres of *Multiceps serialis* and *Hymenolepis diminuta*, reported hook movement identical to that of *A* (*E.*) *procyonis*. However, they failed to state the relationship of these movements to onchosphere locomotion. Ogren (1956, 1957) reported that only the median hooks function efficiently in the movement of the onchosphere of *Mesocestoides corti* and *Mathevotaenia symmetrica*. Lateral hook movements were reported as secondary and occa-

sionally asynchronous.

Cysticercoid development of *Mathevotaenia symmetrica* and *M. deserti* has been described in the works of Rentdorff and Millemann previously cited and is almost identical to that of *A.* (*E.*) *procyonis*. The infective cysticercoid of *A.* (*E.*) *procyonis* may be differentiated from the other two by its larger size and by the fact that development to this stage requires 10 days, while the other two species require 16 to 18 days. Gravid proglottids of *A.* (*E.*) *procyonis* are first passed with the feces of the definitive host in 12 days, while *M. deserti* and *M. symmetrica* require 22 to 23 days. Membrane enclosed cysticercoids of the type encountered in this study were also reported by Rentdorff and Millemann. The significance of this membrane has not yet been determined. Changes in the size of cysticercoids after they have reached the infective stage have not been reported in other linstowid life histories. Such changes in *A.* (*E.*) *procyonis* may be associated with the longer time required for growth to the adult stage when 114-day-old cysticercoids were fed to the raccoon.

## SUMMARY

1. The incidence of *Atriotaenia* (*Ershovia*) *procyonis* in raccoons (*Procyon lotor lotor*) from Ohio is 75.8 percent. Twenty-nine animals from six counties were examined.

2. A redescription of the species is presented. This includes an extension of the size range of most structures and a more complete description of the onchosphere and the excretory system of both the cysticercoid and the adult.

3. The life cycle of this cestode has been completed in the laboratory utilizing the flour beetle *Tribolium castaneum* as the intermediate host. Eggs of *A.* (*E.*) *procyonis* contain fully formed onchospheres when passed with the feces of the raccoon. The mouth parts of the beetle probably rupture the egg shell, and the onchospheres, which then "hatch" almost immediately, penetrate the mid-gut of the intestine. Embryos are in the hemocoel of the insect 38 hours after feeding and continue development there. Cysticercoids are mature and infective in 10 days, and gravid proglottids are passed by the raccoon 12 days after ingestion of such cysticercoids.

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## EXPLANATION OF PLATES

All scale values in millimeters.

FIGURE 1. Egg, dorsal view.

FIGURE 2. Onchosphere, dorsal view, showing hooks, glands and cells. Observed under phase microscope.

FIGURE 3. Onchosphere, dorsal view, showing glands, hooks and posterior vesicle. Stained with neutral red.

FIGURE 4. Onchosphere, ventral view, stained with neutral red.

FIGURE 5. Onchosphere, lateral view, dorsal surface uppermost. Stained with neutral red.

FIGURE 6. Onchosphere hooks. A. Ventral hook of lateral pair. B. Dorsal hook of lateral pair. C. Hooks of median pair.

FIGURE 7. Onchosphere after penetration of mid-gut of the beetle, 38 hours. Larva still adherent to muscle layer.

FIGURE 8. Four-day larva from hemocoel of beetle. Central cavity present.

FIGURE 9. Eight-day larva showing sucker primordia, calcareous granules, cyst wall and posterior invagination of excretory bladder.

FIGURE 10. Nine-day larva showing beginning of invagination of scolex. Calcareous granules included.

FIGURE 11. Ten-day, infective cysticercoid showing excretory system. Calcareous granules not shown.

FIGURE 12. Membrane-enclosed cysticercoid, 114 days.

FIGURE 13. Immature specimen from raccoon, 42 hours.

FIGURE 14. Immature specimen from raccoon, 4 days.

FIGURE 15. Immature specimen from raccoon, 6 days.

FIGURE 16. Mature, craspedote proglottid, dorsal view.

FIGURE 17. Gravid proglottid, eggs scattered uniformly throughout parenchyma.

FIGURE 18. Eggs within gravid proglottid, enclosed singly within parenchymatous cavities.

FIGURE 19. Scolex, dorsal view, showing excretory system.

FIGURE 20. Excretory ring in scolex, dorsal view.

FIGURE 21. Excretory bladder of first proglottid formed by germinative region of neck.

FIGURE 22. Excretory system in mature, craspedote proglottids, dorsal view.

FIGURE 23. Blind endings of dorsal excretory ducts in post-mature proglottids.

PLATE I

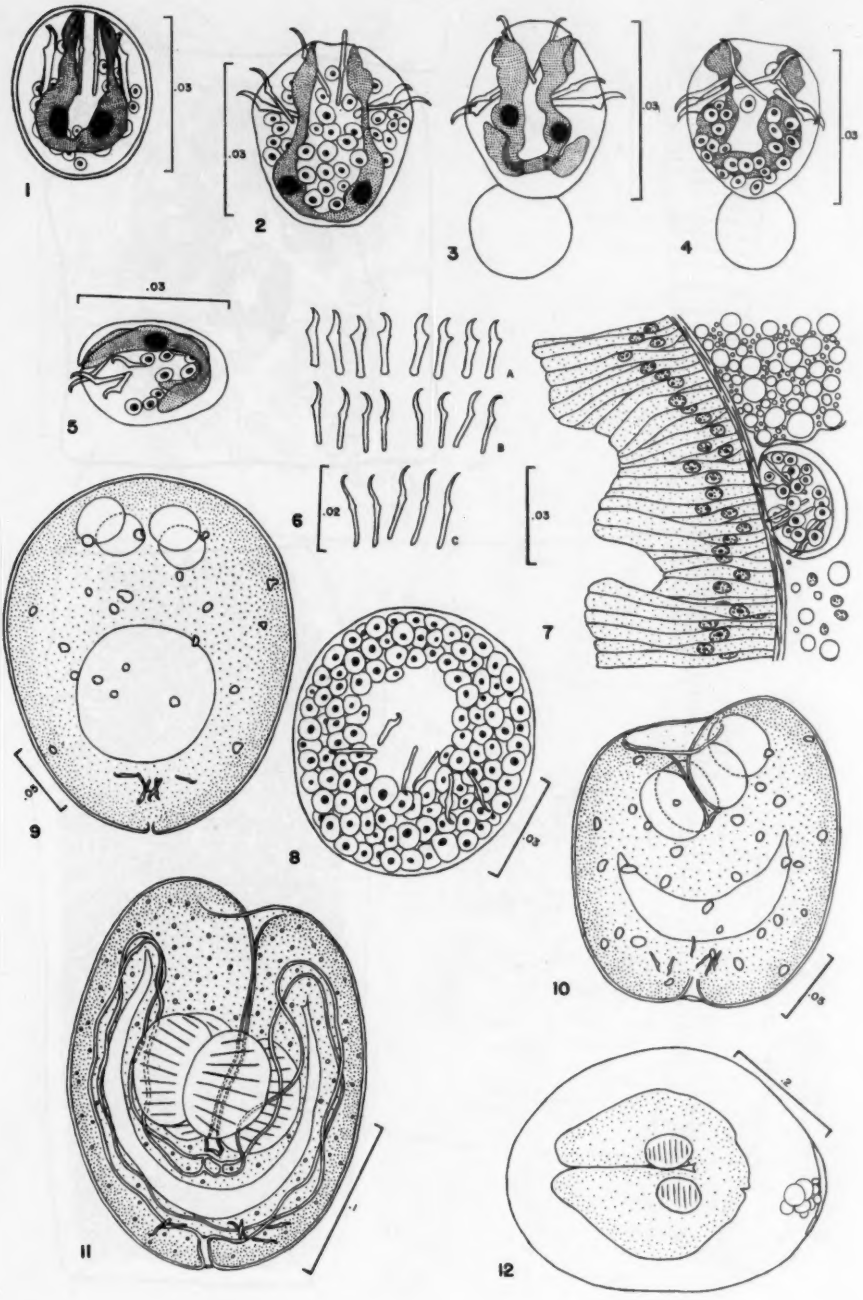
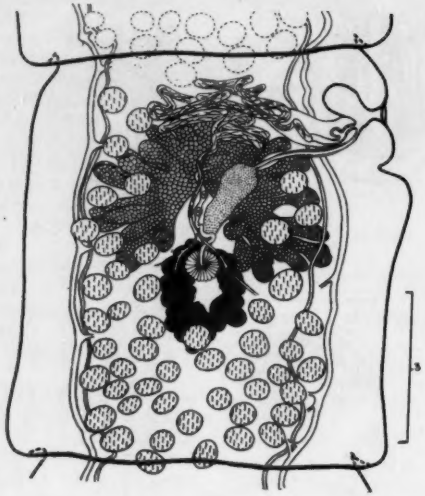
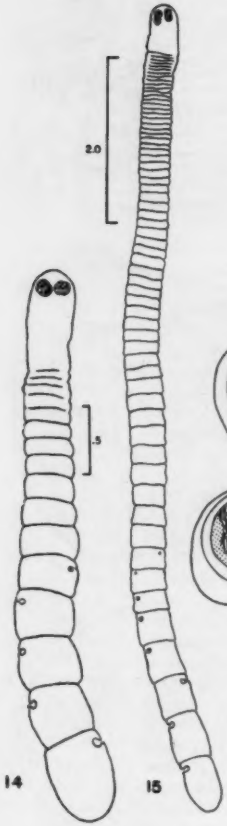
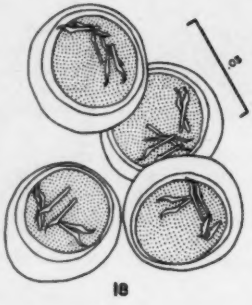


PLATE II



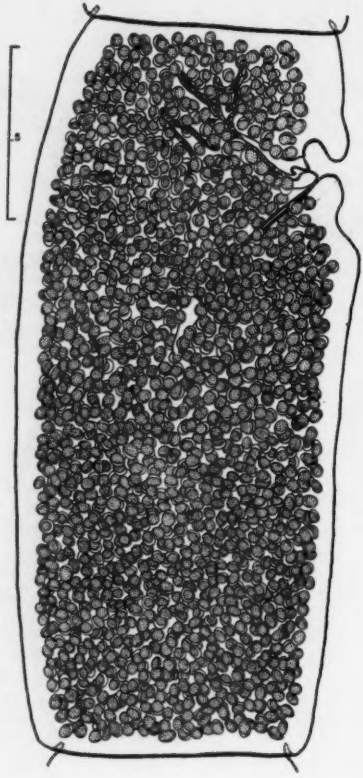
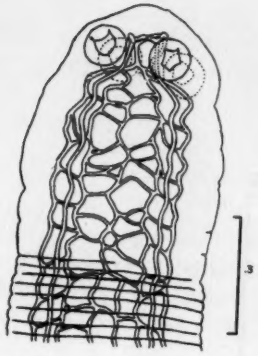



PLATE III

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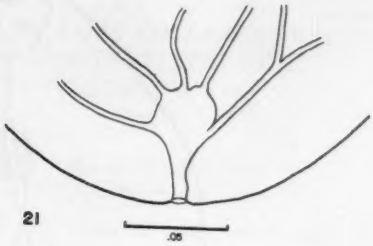


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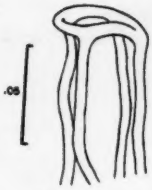


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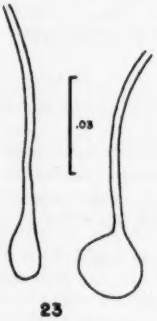


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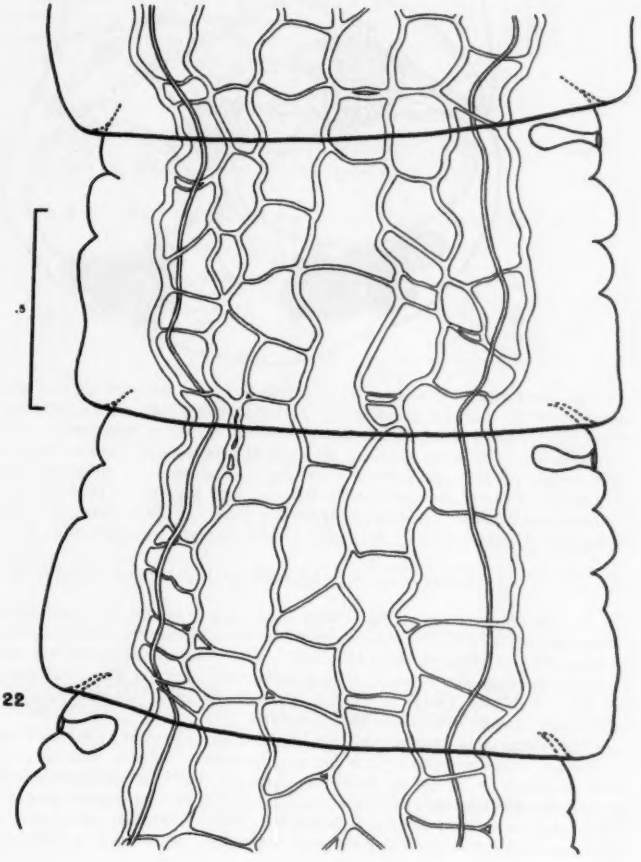


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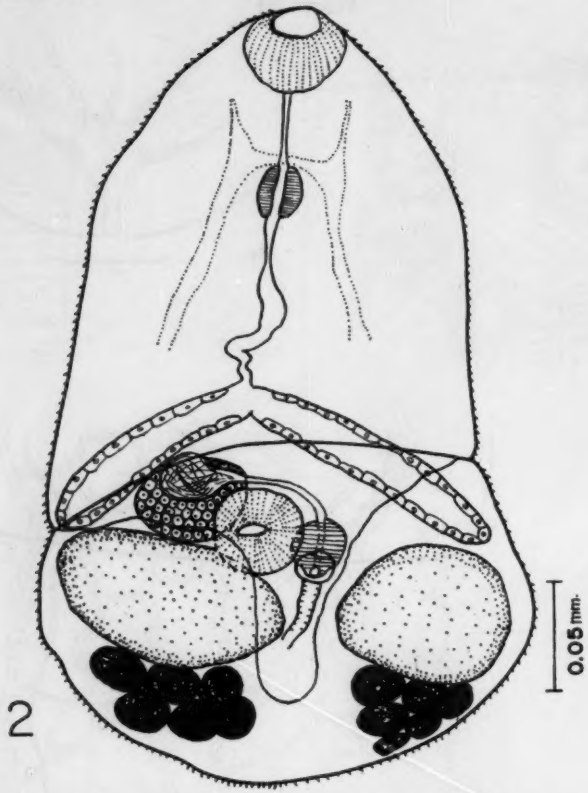


Ten live metacercarial cysts of *Microphallus* from the pink shrimp were fed to a laboratory raised hamster. Careful examination of the digestive tract of the hamster 4 days later did not reveal the presence of any trematodes. Gulls in this area have been found to harbor species of *Microphallus*. The possible role of sea gulls as predators of *Penaeus duorarum* is indicated. Life cycle studies of *Microphallus* sp. from the pink shrimp are presently being conducted in this laboratory.—FRANKLIN SOGANDARES-BERNAL AND ROBERT F. HUTTON, *Florida State Board of Conservation Marine Laboratory, Maritime Base, St. Petersburg, Florida.* Supported in part by a grant (E-2008) from U. S. Public Health Service. Contribution No. 17.

## THE CHROMOSOMES OF *DIPHYLLOBOTHRIUM URSI*

No chromosome studies have been done on any of the Dibothriocephalidae. So far as the writer knows, only one chromosome study on a tapeworm inhabiting humans has been made (Jones, 1957, J. Parasit. **43**: 115–116). The present paper reports chromosome studies on *Diphyllobothrium ursi* Rausch, 1954, a tapeworm naturally infecting bears on Kodiak Island in the Gulf of Alaska (Rausch, 1954, J. Parasit. **40**: 540–563) for details. This tapeworm also infects man, and so is of public health interest.

The tapeworms sent me by Dr. Robert Rausch of the Arctic Health Research Center, Anchorage, Alaska, were from a dog experimentally infected with plerocercoids. The material used consisted of 3 worms fixed in Carnoy's fluid and immediately shipped by air. The preliminary staining was in Feulgen's reagent, but this proved to give a rather weak reaction. Subsequent staining in Cooper and Brink's (1931, Proc. Nat. Acad. Sci. **17**: 334–338) carmine and hematoxylin combination gave very fine results on oocytes undergoing meiosis. The uterus

(*continued on p. 384*)

# THE ROLE OF BILE SALTS IN THE BIOLOGY OF TAPEWORMS. II. FURTHER OBSERVATIONS ON THE EFFECTS OF BILE SALTS ON METABOLISM*

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In a previous paper (Rothman, 1958) it was shown that bile salts inhibit the metabolism of *Hymenolepis diminuta* and *Oochoristica symmetrica.* With this finding as a basis, further experiments were performed with other species of tapeworms to determine how their metabolism is affected.

## MATERIALS AND METHODS

The strobilae of *H. diminuta* were reared in Wistar rats and golden hamsters; *Hymenolepis citelli,* in golden hamsters; *Taenia taeniaeformis,* in domestic cats; and the larvae of *Taenia crassiceps,* in the peritoneal cavity of Princeton Swiss mice. The methods used were the same as those of the previous study (Rothman, 1958).

## RESULTS

The strobilate phase of *H. diminuta* from hamsters shows a response to sodium taurocholate resembling that of the strobilate phase from rats (Table I). There is

TABLE I. *The effects of sodium taurocholate on the anaerobic fermentation of* H. diminuta *from hamsters.*

| | pH 7.0 | | | pH 7.4 | | |
|---|---|---|---|---|---|---|
| | $Q_{acid}$ | Mean % inhibition | Number of determinations | $Q_{acid}$ | Mean % inhibition | Number of determinations |
| A | 1.6 ± 0.3 | — | 7 | 1.4 ± 0.1 | — | 8 |
| B | 21.5 ± 1.5 | — | 4 | 23.8 ± 0.7 | — | 4 |
| | | | 0.1% taurocholate | | | |
| E | 11.7 ± 0.7 | 45.6 | 3 | 16.7 ± 0.2 | 30.0 | 3 |
| | | | 0.3% taurocholate | | | |
| E | 10.8 ± 0.2 | 49.8 | 4 | 14.5 — | 39.2 | 2 |
| | | | 0.5% taurocholate | | | |
| E | 9.7 ± 0.4 | 55.0 | 3 | 16.3 ± 0.8 | 31.5 | 3 |

The $Q_{acid}$ was determined from the gas evolved in the second 20-minute period after the final addition. A—Endogenous; B—Glucose only (control group for all experiments); E—Bile salt and glucose added together.

an inhibition both at pH 7.4 and at pH 7.0, but, as with *H. diminuta* from rats, the inhibition is greater at pH 7.0 than at pH 7.4. There is a small difference between the responses of worms from different hosts; *H. diminuta* from hamsters are inhibited to a slightly greater extent at pH 7.0 in 0.1% taurocholate than are worms from rats. Further, the endogenous metabolism, or the metabolism with added

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glucose, is slightly lower in worms from hamsters than in worms from rats (Rothman, 1958) at both pH 7.0 and 7.4.

Bile salts inhibit the acid production of *H. diminuta* when glucose is used as a substrate. Since *H. diminuta* can metabolize galactose (Laurie, 1957), it seemed pertinent to test the effects of bile salts on the metabolism of this substrate. As observed by Laurie (1957), the data (Table II) indicates that galactose is metab-

TABLE II. *The effects of sodium taurocholate on the anaerobic fermentation of galactose by* H. diminuta *from rats.*

| | pH 7.0 | | | pH 7.4 | | |
|---|---|---|---|---|---|---|
| | $Q_{acid}$ | Mean % inhibition | Number of determinations | $Q_{acid}$ | Mean % inhibition | Number of determinations |
| A | 2.6 ± 0.5 | – | 12 | 2.4 ± 0.1 | – | 6 |
| B | 7.2 ± 1.2 | – | 4 | 9.5 ± 1.5 | – | 5 |
| | | | 0.1% taurocholate | | | |
| E | 6.1 ± 0.1 | 0 | 3 | 8.3 ± 0.7 | 0 | 3 |
| | | | 0.5% taurocholate | | | |
| E | 8.0 ± 0.1 | 0 | 3 | 8.7 ± 0.5 | 0 | 3 |

The $Q_{acid}$ was determined from the gas evolved in the second 20-minute period after the final addition. A—Endogenous; B—Galactose only (control group for all experiments); E—Bile salt and galactose added together.

olized at a lower rate than glucose by *H. diminuta* from rats. Moreover, there is no demonstrable inhibition of acid production in the presence of bile salts when galactose is being fermented.

Some experiments were performed to determine whether the strobilate phase of *T. taeniaeformis* and *H. citelli,* and the larval phase of *T. crassiceps,* respond to taurocholate in a manner resembling that observed with *H. diminuta.* The data in table III indicates that *H. citelli,* a worm morphologically similar to *H. diminuta,*

TABLE III. *The effects of sodium taurocholate on the anaerobic fermentation of* H. citelli.

| | pH 7.0 | | | pH 7.4 | | |
|---|---|---|---|---|---|---|
| | $Q_{acid}$ | Mean % inhibition | Number of determinations | $Q_{acid}$ | Mean % inhibition | Number of determinations |
| A | 1.7 ± 0.1 | – | 10 | 1.7 ± 0.4 | – | 10 |
| B | 5.1 ± 0.6 | – | 12 | 8.0 ± 0.7 | – | 17 |
| | | | 0.1% taurocholate | | | |
| C | 3.8 ± 0.4 | 0 | 6 | 7.0 ± 1.2 | – | 5 |
| E | 3.8 ± 0.4 | 0 | 6 | 6.5 ± 0.8 | 0 | 5 |
| | | | 0.3% taurocholate | | | |
| C | 4.9 ± 0.7 | 0 | 3 | 7.1 ± 0.9 | 0 | 3 |
| E | 5.5 ± 0.6 | 0 | 3 | 7.4 ± 0.4 | 0 | 4 |
| | | | 0.5% taurocholate | | | |
| C | 3.8 ± 0.2 | 0 | 6 | 6.1 ± 0.5 | 0 | 10 |
| E | 4.8 ± 0.2 | 0 | 6 | 6.0 ± 0.2 | 0 | 6 |

The $Q_{acid}$ was determined from the gas evolved in the second 20-minute period after the final addition. A—Endogenous; B—Glucose only (control group for all experiments); C—Bile salt added 20–30 minutes before glucose; E—Bile salt and glucose added together.

has endogenous metabolic rates equal to, and glucose fermentation rates lower than those of *H. diminuta* from hamsters, approximating the galactose fermentation rates of *H. diminuta* from rats. A difference in uninhibited glucose fermentation at the two pH's is quite apparent for *H. citelli,* whereas *H. diminuta* does not show

such a marked difference except in the case of inhibition with a bile salt. Taurocholate produces no significant inhibition of the acid production of *H. citelli* at pH 7.0 or 7.4.

The acid production of *T. taeniaeformis* is not altered by the addition of glucose at either pH and is unaffected by the addition of bile salts in the presence or absence of glucose at pH 7.0. At pH 7.4 there is an apparent stimulation of endogenous metabolism in the presence of bile salts (Table IV).

TABLE IV. *The effects of 0.5% sodium taurocholate on the anaerobic fermentation of* T. taeniaeformis.

| | pH 7.0 | | pH 7.4 | |
|---|---|---|---|---|
| | Q acid | Number of determinations | Q acid | Number of determinations |
| A | 2.5 ± 0.1 | 5 | 2.0 ± 0.2 | 6 |
| B | 2.8 — | 2 | 2.0 ± 0.2 | 3 |
| C | 2.8 — | 2 | 2.9 ± 0.1 | 3 |
| D | 3.4 ± 0.3 | 4 | 3.2 ± 0.2 | 6 |

The $Q_{acid}$ was determined from the gas evolved in the second 20-minute period after the final addition. A—Endogenous; B—Glucose only; C—Bile salt only; D—Bile salt and glucose.

The acid production of *T. crassiceps* larvae is the same in the presence of exogenous glucose as in its absence, but the acid production is stimulated upon addition of bile salts in the presence or absence of glucose, the glucose having no apparent effect even in combination with the bile salt (Table V).

TABLE V. *The effects of sodium taurocholate on the anaerobic metabolism of* T. crassiceps *larvae.*

| | pH 7.4 | | |
|---|---|---|---|
| | Q acid | Mean % stimulation | Number of determinations |
| A | 1.5 ± 0.2 | — | 12 |
| B | 1.8 ± 0.1 | 0 | 9 |
| C | 3.1 ± 0.1 | 106.8 | 3 |
| D | 2.7 ± 0.2 | 80.0 | 3 |
| E | 3.4 ± 0.2 | 121.5 | 6 |

The $Q_{acid}$ was determined from the gas evolved in the second 20-minute period after the final addition. A—Endogenous; B—Glucose only; C—0.2% taurocholate only; D—0.2% taurocholate with glucose; E—0.5% taurocholate with glucose.

Bile salts are normal constituents of the bile secreted into the intestine. Therefore, it seemed desirable to determine whether whole bile would inhibit the metabolism of *H. diminuta,* and whether the inhibition, if observed, is altered by the change in pH. At the time of these experiments autopsies were being performed, almost daily, on the cottontail rabbit, *Sylvilagus floridanus.* A sufficient quantity of whole bile was available to use in two trial experiments with *H. diminuta.* Table VI indicates that there is an inhibition of glucose fermentation with diluted whole rabbit bile at pH 7.0, but none at pH 7.4. The inhibition at pH 7.0 is at about the level of inhibition of 0.1% taurocholate. The bile of the rabbit, therefore, inhibits the glucose fermentation of *H. diminuta* at low concentrations; moreover, there was an inhibition at pH 7.0, in the presence of whole bile, indicating that some substance is acting as an inhibitor at this pH, but not at pH 7.4. This substance may be a bile salt; sodium glycocholate and sodium taurocholate are present in rabbit bile, glycocholate predominating in quantity (Dukes, 1955).

TABLE VI. *The effects of diluted whole bile from* Sylvilagus floridanus *on the anaerobic fermentation of* H. diminuta.

| | pH 7.0 | | | pH 7.4 | | |
|---|---|---|---|---|---|---|
| | $Q_{acid}$ | Mean % inhibition | Number of determinations | $Q_{acid}$ | Mean % inhibition | Number of determinations |
| A | $2.6 \pm 1.7$ | — | 12 | $2.4 \pm 0.1$ | — | 6 |
| B | $27.3 \pm 0.8$ | — | 15 | $29.9 \pm 0.3$ | — | 10 |
| C | 18.3 — | 32.8 | 2 | 27.9 — | 0 | 2 |
| D | 21.9 — | 20.0 | 2 | 29.3 — | 0 | 2 |

The $Q_{acid}$ was determined from the gas evolved in the second 20-minute period after the final addition. A—Endogenous; B—Glucose only (control group for all experiments); C—Bile diluted 1 : 10, added 20 minutes before glucose; D—Bile diluted 1 : 20, added 20 minutes before glucose.

## DISCUSSION

The effects of bile salts on various organisms was discussed in an earlier paper (Rothman, 1958). It may be mentioned that various tapeworm species differ in responses to bile salts, as evidenced by their metabolic activity and patterns of excystment (Rothman, in press).

Further studies with other species of cestodes is essential to determine whether the bile salts influence metabolism and under what special conditions of potential host physiology these influences of the bile salts might be limiting environmental factors. The bile salts are one of many factors which may combine to effect host specificity of tapeworms.

The work of Goodchild (1958), which indicates that bile salts are essential for the normal development of *H. diminuta* in the intestine of the rat, might seem to contradict the finding that bile salts inhibit the metabolism of *H. diminuta*. This, however, is not necessarily so, since the absence of bile from the small intestine (though it is rerouted to the colon) undoubtedly upsets the normal physiology of the gut, and the tapeworm may be adversely affected by secondary factors attributable to the absence of bile rather than due to the direct effect of bile alone.

With available data, it cannot be ascertained what factors are involved in the varied responses of different tapeworms to the bile salts. It is possible that the stimulation noted in the *T. crassiceps* larvae is due to increased muscular activity involved in the evagination process that the larvae undergo upon the addition of bile salts, the acid formation arising from endogenous sources.

The absence of inhibition of galactose fermentation with bile salts by *H. diminuta* may indicate the operation of separate systems for the permeation of the carbohydrate substrates glucose and galactose. In support of this, Laurie (1957) reported that acriflavin inhibits glucose fermentation by *H. diminuta,* but does not affect galactose fermentation.

The inhibition of glucose fermentation by *H. diminuta* with diluted whole rabbit bile supports the hypothesis that bile can in vivo exert limiting effects which may, under certain conditions, influence establishment in a potential host.

## SUMMARY

It has been shown that the bile salt inhibition of glucose fermentation by *H. diminuta* from hamsters is similar to the pattern of bile salt inhibition demonstrated by Rothman (1958) for *H. diminuta* from rats and *Oochoristica symmetrica* from mice. In contrast to this, the galactose fermentation of *H. diminuta* is unaffected by bile salts.

The response of glucose fermentation of different tapeworms to the presence of bile salts varies as evidenced by the fact that, although the glucose fermentation of some is inhibited, the glucose fermentation of others, such as *H. citelli* and *T. taeniaeformis,* is unaffected.

The glucose fermentation of the larval stage of *T. crassiceps* is unaffected by bile salts, but bile salts apparently stimulate endogenous fermentation.


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## RESEARCH NOTE

### TREMATODE INFECTIONS IN TERRESTRIAL MOLLUSKS OF MINNESOTA

During the summer of 1957, a limited parasitological survey was made of the terrestrial mollusks of the Lake Itasca, Minnesota, area to obtain information as to the infection with trematodes. Mollusks were collected along the perimeter of Lake Itasca at several stations chosen from a variety of habitats: (1) Predominantly evergreen stands; (2) predominantly hardwood stands, (3) mixed hardwood and evergreen stands, (4) relatively open plots of grasses, (5) marshy areas along the headwarters of the Mississippi River. Collecting was accomplished by sifting litter and by examining habitat materials *in situ.* The accompanying table incorporates the essential data derived from the study.

A total of 533 mollusks representing 13 genera and species were examined. Of the 8 specimens that carried trematode infections, 1 harbored rediae and many cercariae, 1 had metacercariae, and each of the other 6 contained from 1 to 3 mesocercariae. The predominantly hardwood stands yielded the largest number and variety of mollusks as well as the highest percentage of infection.

Other parasites that were found but are not discussed in this report were (in descending order of prevalence) as follows: (1) Nematodes, (2) protozoa (tentatively identified as *Tetrahymena* sp.), (3) cestodes (cysticercoids).

TABLE I. *Infection data relating to trematodes in terrrestrial mollusks.*

| Species | Number examined | Number infected | Percent infected |
|---|---|---|---|
| *Anguispira alternata* | 36 | 2 | 5.6 |
| *Carychium* sp. | 9 | 0 | 0.0 |
| *Cionella lubrica* | 31 | 0 | 0.0 |
| *Deroceras* sp. | 6 | 0 | 0.0 |
| *Discus cronkhitei* | 116 | 1 | 0.9 |
| *Euconulus* sp. | 20 | 0 | 0.0 |
| *Helicodiscus parallelus* | 10 | 0 | 0.0 |
| *Retinella electrina* | 101 | 0 | 0.0 |
| *Strobilops labyrinthica* | 24 | 0 | 0.0 |
| *Vallonia gracilicosta* | 37 | 1 | 2.7 |
| *Vitrina limpida* | 8 | 0 | 0.0 |
| *Zonitoides arboreus* | 121 | 4 | 3.3 |
| *Zonitoides nitidus* | 14 | 0 | 0.0 |
| Total no. examined 533 | Total no. infected 8 | % of total infected 1.5 | |

This work was supported by the University of Minnesota and by a National Science Foundation grant-in-aid.—JAMES A. PATTEN, *Middle Tennessee State College, Murfreesboro, Tennessee.*

(*continued from p. 378*)

and testes were dissected under a wide field microscope following Feulgen staining, and mounted directly in the Cooper and Brink's stain. It was unfortunate that the only really clear figures obtained were those of meiosis in the female nucleus of the egg. Some preliminary work on an unnamed species of *Diphyllobothrium* from the same area, sent me by Dr. Rausch, indicates that immature ovaries may be an excellent source of somatic mitoses.

Figure 1 is a photomicrograph of the female meiotic chromosomes. Figure 2 is a diagramatic drawing of the same figure made by inking a print and subsequently washing out the photographic image with Farmer's reducer. Each is shown at a magnification of about 2000×.

The haploid chromosome number is 9. These may be seen in Figures 1 and 2. Accurate counts were made many times in meiosis in the female nucleus. Approximate counts in the clumped metaphases of testicular and somatic tissues agreed with this. Of the 9 pairs of chromosomes, 4 are very small.—GORDON B. WOLCOTT, *Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases, P. O. Box 717, Columbia, South Carolina.*

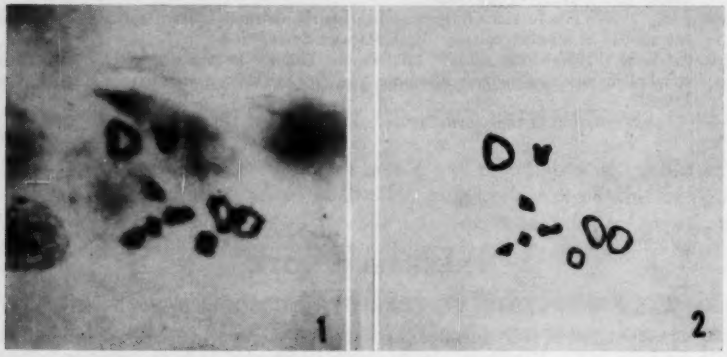


FIGURE 1. Chromosomes at first metaphase of meiosis in an oocyte.

FIGURE 2. Diagrammatic representation of the same set of chromosomes, made as described in the text.

## NOTES ON THE DISTRIBUTION OF THE LEECH, *MYZOBDELLA LUGUBRIS* LEIDY, AND ITS ASSOCIATION WITH MORTALITY OF THE BLUE CRAB, *CALLINECTES SAPIDUS* RATHBUN

Mortality of marine animals in the field is frequently brought to our attention by Conservation Agents of our Department. In this connection, a leech was found associated with mortality of the blue crab, *Callinectes sapidus* Rathbun, in Bulow Creek, Volusia County, Florida, on 15 September 1957. Agent A. F. Dickerson observed leeches attached to and "penetrating" the carapace of some blue crabs. According to Agent Dickerson, in over 50% of 30 crabs, leeches were protruding, expanding and contracting, through holes in the dorsal aspect of the carapace. The leeches were observed protruding from the holes only upon removal of the crab from the water. Seven crabs were preserved in formalin. This material, along with other biological specimens, was transported to the Florida State Board of Conservation Marine Laboratory by General Agent H. C. Mackery, Jacksonville, Florida.

Examination of the material collected by Agent Dickerson revealed the presence of 32 leeches attached to the carapace near the base of the legs (Fig. 1) as well as on the dorsal aspect of the carapace and on the bottom of the container. The dorsal aspect of 1 crab was thrice perforated (Fig. 2). Leeches were not actually observed boring the 3 perforations, but instead utilizing the holes. The 3 perforations may have been produced by injury, some other animal or a disease. It is also possible that the leeches may have made these perforations during ecdysis when the carapace was in a soft condition.

Specimens of the leech were sent to Dr. J. Percy Moore who identified them as *Myzobdella lugubris* Leidy. Dr. Moore stated, "I doubt, but do not know certainly, if the leeches damage

(*continued on p. 404*)

# A REVISION OF THE GENUS *HAPLOMETRANA* LUCKER, 1931 (TREMATODA: PLAGIORCHIIDAE), WITH NOTES ON ITS DISTRIBUTION AND SPECIFICITY*

J. ALLAN WAITZ

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While examining specimens of *Rana pretiosa* Baird and Girard from Idaho, the author recovered a number of helminths of the genus *Haplometrana* Lucker, 1931. In attempting to identify the species involved, the variability of the individuals and the similarities of the 2 described species became apparent. After studying the distribution of the host, it appeared that the 2 described species of *Haplometrana* were sampled from hosts at 2 ends of a cline, in the center of which was Idaho. It seemed reasonable to assume that the parasites as well were 2 ends of a cline and the 2 species were synonymous. In order to prove or disprove these suppositions, a wide-spread collection was made and a large number of worms were studied. The results of this study are presented in this paper.

The genus was named and described by Lucker (1931) the type species being *H. intestinalis* from the small intestine of *Rana pretiosa* from Bothell, Washington. Olsen (1937a) described a new species, *H. utahensis*, from the small intestine of *Rana pretiosa* from Springville, Utah, and studied its life cycle. Olsen (1937b), in his study of the subfamily Plagiorchiinae, revised the generic diagnosis and again differentiated between the 2 species in a key.

## MATERIALS AND METHODS

Forty-six specimens of *Rana pretiosa* from 9 localities and 19 specimens of a hybrid cross of *R. pretiosa* and *R. sylvatica* LeConte from 3 localities were examined for parasites. The hosts were examined as soon after collecting as possible. The intestinal tracts of the hosts were opened in 0.7% sodium chloride solution, and the specimens were relaxed by shaking or by refrigeration overnight. Specimens were fixed under a cover glass with a slight amount of pressure. Fixatives used were Gilson's and A.F.A. All specimens were stored in 70% ethyl alcohol. Stains used were Ehrlich's hematoxylin, Semichon's acetic carmine and alum cochineal.

Three worms were imbedded in paraffin and sectioned at 8 microns. The sections were stained with Ehrlich's hematoxylin.

Altogether, 338 flukes were obtained. Of the hybrid hosts, 9 of 19 (47.3%) were infected with 98 worms, varying between 1 and 23 worms per individual. Of the typical hosts, 31 of 46 (67.4%) were infected with 240 worms, ranging from 1 to 27 each.

## RESULTS

Olsen (1937a) noted certain characters used to differentiate this species from *H. intestinalis*. These differences were noted in $\frac{\text{length}}{\text{width}}$ ratio, nature of the acetabulum, seminal vesicle, position of the testes, vas efferens, vas deferens, Laurer's canal and the yolk reservoir.

A study of specimens loaned to the author by Drs. Lucker and Olsen, and 338 specimens collected by the author shows that *H. utahensis* is synonymous with *H. intestinalis*. The differences noted by Olsen may be taken in order.

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* This investigation was supported in part by a research grant from the Northwest Scientific Association.

*Length / Width ratio.* This was the first of the differences noted by Olsen. His specimens showed a length of 5 mm and a width of 0.7–0.8 mm, the width being contained in the length 5 to 7 times. Lucker's specimens were 5 mm long and 0.48–0.63 mm in width, the length:width ratios being from 8 to 10. Fifty of the specimens collected by the author averaged 4.034 mm long (range 2.57–6.97), and 0.478 mm wide (range 0.28–0.76); $\frac{\text{length}}{\text{width}} = 8.642$ (range 5.13–12.26). It is obvious that there is a great deal of variation in the dimensions of the worms and that the use of the $\frac{\text{length}}{\text{width}}$ ratio as a specific difference is invalid.

*Acetabulum.* Olsen noted that his specimens did not have a distinct conical muscular mass dorsal to the acetabulum as was the case in *H. intestinalis.* Of 4 specimens of *H. intestinalis* loaned to the author by Dr. Lucker, only 1 showed this character. All specimens did show, however, a varying degree of condensed tissue dorsal to the acetabulum (Fig. 1). Due to this variation and the fact that the majority of Lucker's specimens failed to show a distinct mass dorsal to the acetabulum, this character should not be used to denote a specific difference.

*Seminal vesicle.* Olsen noted that the seminal vesicle in his specimens was divided into a long posterior and a small round anterior part by a constriction. Lucker did not note this on his specimens. All worms examined in this study, including those lent to the author by Lucker, show this character (Figs. 2, 3, 4, 5). In view of the fact that all specimens showed a bipartite seminal vesicle, this character is invalidated as a specific difference.

*Position of testes.* Olsen's specimens showed the testes to be oblique and less than the intercecal space in diameter. Lucker described his 10 specimens as having testes tandem and filling the intercecal space, often over-lapping the ceca. In the course of this investigation, oblique as well as tandem testes were observed. In addition, all variations between these two conditions were noted. Variations in the diameter of the testes were also observed and were related to their placement. Minor variations in position, as evidenced here, are often due to the rigors of fixation. Position and size of testes appear too variable to be used for specific differences.

*Vas efferens.* Olsen stated that in his specimens the vas efferens from the posterior testis always passes mesad between the ovary and the seminal receptacle, while in *H. intestinalis,* it passes caudad to the seminal receptacle. Lucker actually stated that "in specimens in which the vas efferens may be traced anteriorly, the position of the duct varies considerably."

In only a few specimens was it possible to trace definitely the anterior course of this duct. In most of these, the duct passed mesad, between the ovary and seminal receptacle. In 1 specimen, it appeared to pass caudad to the seminal receptacle.

*Vas deferens.* Lucker noted a sac or reservoir on the vas deferens shortly before the cirrus pouch is reached. Olsen stated that this was not present in his specimens. In some of my specimens there appeared to be a small dilation in the duct just posterior to the cirrus pouch. This character is too difficult to discern, and probably too variable to distinguish species.

*Laurer's canal.* Lucker diagrammed Laurer's canal as entering the body of the

seminal receptacle. Olsen found it emptying into the stem of the seminal receptacle. Sections of specimens show Laurer's canal joining the stem of the seminal receptacle (Fig. 6).

It is evident that the differences which Olsen used to separate *H. utahensis* from *H. intestinalis* are not valid. This situation leaves but one logical course, to reduce *H. utahensis* to synonomy. *Haplometrana* is a highly variable genus and an emended generic diagnosis is offered which encompasses these variations.

### Genus *Haplometrana* Lucker, 1931

*Generic diagnosis:* Plagiorchiinae: Slender worms, bluntly rounded at both extremities, and flattened dorso-ventrally. Cuticle spinose, oral sucker subterminal. Esophagus long and slender, intestinal ceca may vary in length, but always extend to near posterior end of body. Cirrus protrusible, cirrus pouch long, extending dorsal to and beyond posterior margin of acetabulum. Seminal vesicle occupies posterior half of cirrus pouch and is divided into a long posterior and a short anterior portion by a constriction. Testes oval, either tandem or oblique, dorsal to both rami of uterus. Vitellaria are lateral and in unorganized chains extending from near the level of the acetabulum posteriorly to behind posterior testis. Excretory bladder sac-shaped.

*Type and only species: H. intestinalis* Lucker, 1931. Intestine of *Rana pretiosa,* Washington State.

### DISTRIBUTION AND SPECIFICITY

The species appears to be highly host specific. A single specimen was found in the intestine of *Bufo boreas* Baird and Girard, but this is thought to be accidental. Of the 46 specimens of *Rana pretiosa* from 9 localities, 67.4% were infected. A smaller percentage of infection, 47.3%, was noted in the crosses of *R. pretiosa* and *R. sylvatica* from 3 localities. *R. pipiens,* captured from the same ponds that harbored infected *R. pretiosa,* were not infected. Hosts were infected in all localities sampled in northern and central Idaho. The distribution of the parasite is evidently dependent on the distribution of *R. pretiosa.*

### SUMMARY

A total of 338 specimens of flukes of the genus *Haplometrana* were obtained from 65 frogs. The differences noted by Olsen in his description of *H. utahensis* are invalidated and the species is reduced to synonomy. An emended generic diagnosis of *Haplometrana* is given with the type and only species being *H. intestinalis* Lucker, 1931. The worm is highly variable, but is extremely host specific. With the exception of 1 toad, no amphibians other than *Rana pretiosa* were infected. The distribution is apparently limited by the distribution of this host.

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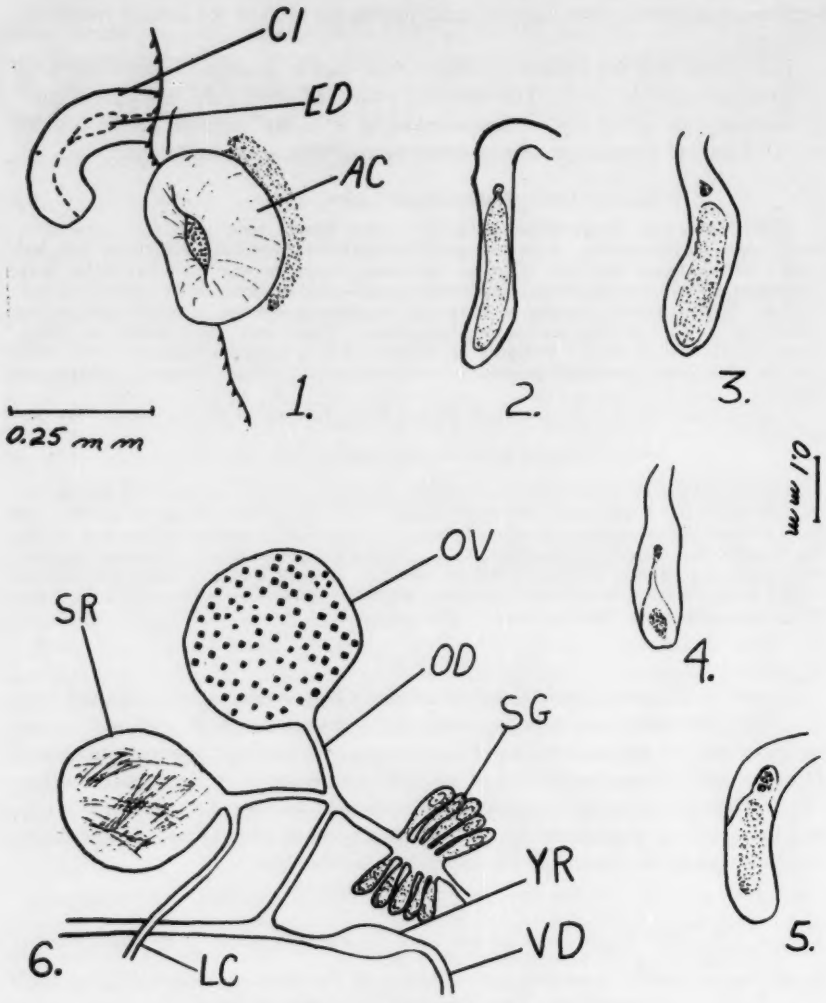
PLATE I




EXPLANATION OF FIGURES

FIGURE 1. Lateral view of acetabular region of *Haplometra intestinalis* showing mass of condensed tissue dorsal to the acetabulum. Camera lucida.

FIGURES 2, 3, 4 and 5. Views of seminal vesicles of *H. intestinalis* showing its bipartite nature and some variations. Camera lucida.

FIGURE 6. Diagram of female reproductive system of *H. intestinalis*.

AC—acetabulum
CI—cirrus
ED—ejaculatory duct
LC—Laurer's canal
OD—oviduct
OV—ovary
SG—shell gland
SR—seminal receptacle
VD—vitelline duct
YR—yolk reservoir

# INDUCED GAMETOGENESIS IN A MONOGENETIC TREMATODE, *POLYSTOMA STELLAI* VIGUERAS, 1955*

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In the first comprehensive treatise on the endocrine organs and secretions of invertebrate animals, Koller (1929) reviewed the observations of earlier authors. The following decade witnessed the important monographic accounts of de Lerma (1934; 1936), Koller (1937), Hanström (1937; 1939), and von der Wense (1938). Koller and Meyer (1930) reported that the hormone from the eye-stalks of crustaceans *Crangon vulgaris* and *Praunus inermis* caused expansion of the chromatophores of the fishes, *Gobius ruthensparri* and *Pleuronectes platessa.* The results were confirmed by Kropp and Perkins (1933). Abramowitz (1936) reported that the hormone intermedin, prepared from the pituitary glands of sheep, expanded the chromatophores of the fiddler crab, *Uca,* after the pigment had been contracted by the removal of the eye-stalks. He stated (p. 523), "The vertebrate pigmentary hormone is thus capable of evoking responses of invertebrate chromatophores, just as the crustacean hormone is capable of acting on the chromatophores of vertebrates as first demonstrated by Koller and Meyer and by Kropp and Perkins." Schmidt (1935) reported more rapid growth and earlier sexual maturity of the fresh-water polychaete, *Lycastis ranauensis,* when thyroid, anterior pituitary, and thymus preparations were added to the culture media. Reporting the investigation, Feuerborn (1936) stated (p. 139), "Zusammenfassend kann gesagt werden, dass die Versuche an *Lycastis* im Gegensatz zu bisherigen Versuchen an Wirbellosen zeigen, dass dieses Object von Wirbeltierhormonen im engeren und weiteren Sinne nach verschiedener Richtung und in z.T. hohem Masse beeinflusst wird." The results were accepted by Hanström (1939) who stated (p. 11), "The action of the gonadotropic hormone of the hypophysis was for the first time established in invertebrates."

Further studies in the past 20 years have demonstrated the presence and functions of hormone substances in plants and have established clearly that endocrine secretions regulate rates of physiological processes in both vertebrate and invertebrate animals. It is well-established that growth and molting of insects are directed and controlled by endocrine secretions. Cleveland (1957) reported correlation between molting of the wood-feeding roach, *Cryptocercus,* and the successive sexual processes in members of 10 genera of protozoans which live in the intestine of this insect. The observation was confirmed by Nutting and Cleveland (1958) on evidence from cervical ligatures, glandular extirpations, and transfaunation experiments. The procerebral pars intercerebralis was designated as the regulatory center. However, studies on the effects of the hormones of vertebrates on invertebrates and of extracts of invertebrate tissues on vertebrate animals have led to divergent and often conflicting results. In certain instances, effects attributed to hormones have been interpreted by other investigators as merely the results of more or less favorable nutritional conditions, or of irritation, or of intensification of normal activities.

* Received for publication January 7, 1959.

Magnus (1903) reported that application of adrenalin to a local portion of the ventral nerve cord of *Sipunculus nudus* caused contraction of adjacent muscles, to which a reader noted, "so would acetic acid." Discussing the endocrine control of morphogenesis and metamorphosis of insects, Williams (1953) declared (p. 149), "Indeed, it is an extraordinary fact that, aside from neurohumors, none of the potent hormones of vertebrates appear to have any clear-cut effect on any invertebrate." Recently, Gilbert and Schneiderman (1958) reported the induction of precocious molting of immature pupae of the moth, *Antheraea polyphemus,* by injection of extracts of adrenal cortex from beef. It was suggested that the effective component is a steroid, perhaps related to the steroids that are concerned with reproductive processes in vertebrate animals.

Monogenetic trematodes of the genus *Polystoma* live in the urinary bladder of amphibians and are strictly hematophagous. They ingest the substances which are circulating in the vascular systems of their hosts and, accordingly, appear to provide excellent material for investigation of the effects of endocrine substances of invertebrates on an invertebrate species. The life-cycle of *Polystoma integerrimum* Fröhlich, 1791, a parasite of the common European frog, *Rana temporaria,* was described by Zeller (1876) after partial and preliminary information had been presented by him (1872) and by other authors. He reported that frogs acquire the worms while tadpoles; that host and parasite develop simultaneously, both becoming sexually mature 3 or 4 years later; that the period of egg-production is relatively short, a few weeks at most; and that the gonads regress after the breeding period. Oviposition by the amphibian and the parasite is concurrent; their larvae occur together in the same pond; the ciliated larvae of the parasite attach to the gills of tadpoles, and when the gills are resorbed at metamorphosis, the worms migrate through the inactive and shortening digestive tract to enter the developing urinary bladder. Zeller reported that if the larvae of the parasite attach to the gills of very young tadpoles, they may develop to sexual maturity on the gills, but that these worms are morphologically very different from those which mature in the urinary bladder and agree closely with *Polystoma ocellatum* Rudolphi, 1819, a species which occurs in the oral cavities of turtles, especially members of the genus *Emys.*

Subsequent authors were inclined to doubt that a monogenetic trematode could produce 2 dimorphic generations, especially when one of them was almost identical morphologically with a related species which lives in the oral cavities of turtles. Caullery, Guyénot and Rivet (1929) and Caullery (1931) noted that the report of Zeller had not been confirmed and indeed had been questioned. Accordingly, Gallien (1935) restudied the biology and life-history of *P. integerrimum.* The worms were found in only *R. temporaria* and appeared to have the same geographic range as that frog. Gallien corroborated the observation of Zeller, that the worms remain juvenile in the urinary bladder for at least 3 years, i.e., until the frog matures. He confirmed and extended other findings of Zeller and made more exact observations on the temporal relations of oviposition by the frog and by the parasite. The larvae from eggs of the bladder-generation were designated gyrodactyloid I, and those from eggs of the gill-generation gyrodactyloid II. Experiments demonstrated that the larvae of the two generations are genetically identical and that nutritive differences determine the course of development. The worms which matured on the gills were characterized as the neotenic generation. They did not migrate and perished when the gills were resorbed at metamorphosis, which in certain experiments was induced

by the use of thyroid. To explain the synchronous sexual maturity of host and parasite, he raised the question whether or not the secretion from the pituitary gland, ingested with blood by the polystomes, might be responsible for the simultaneous sexual maturity of both host and parasite. In subsequent studies, Gallien (1938a; 1938b) investigated the geographic distribution and host-specificity of *P. integerrimum*. *Rana temporaria* and *P. integerrimum* are not present in the region of Toulouse, but a closely related polystome was found in the urinary bladder of *Hyla arborea* var. *meridionalis*. This parasite was named *Polystoma gallieni* by Price (1939).

Paul (1935) noted that species of *Polystoma*, although common and widely distributed in frogs of the eastern hemisphere, had not been reported from species of *Rana* in North America. On the other hand, he described a polystome, which he designated *Polystoma integerrimum nearcticum*, from the urinary bladder, genital ducts, kidneys, and gills of *Hyla versicolor* collected in New York and Connecticut. From an investigation over a period of 3 years, Paul (1938) demonstrated that this polystome has a life-cycle virtually identical with that of *P. integerrimum* in Europe. The concurrent maturity of host and parasite was noted, but no experiments were performed to determine the cause. Price (1939) elevated the subspecies to specific rank.

Other polystomes have since been recorded from North American species of *Hyla*. Caballero and Cerecero (1941) described *Polystoma naevius* from the urinary bladder of *Hyla baudinii* taken near Vera Cruz, Mexico, and Vigueras (1955) described *Polystoma stellai* from a single mature specimen found in the urinary bladder of *Hyla septentrionalis* at Habana, Cuba. The worm was 7.1 mm long; 2.1 mm wide; the posterior haptor was 1.4 mm long by 2.1 mm wide and each sucker measured 0.35 by 0.38 mm in diameter.

In an attempt to determine whether or not the gonadotropic hormone of the pituitary is responsible for the simultaneous sexual maturity of amphibian and parasite, the writer injected a commercial preparation* into juvenile specimens of *Hyla versicolor* which had been collected as tadpoles and maintained for 6 months in the laboratory. As a result of the injections, the gonads increased slightly in size, but none of the animals harbored a polystome, so the purpose of the investigation was not realized.

In the fall of 1954, Dr. James A. Oliver, Curator at the New York Zoological Society, received several half-grown specimens of *Hyla septentrionalis* collected in Florida. He very kindly donated 6 of them for the projected experiment and grateful acknowledgment is hereby made. Two specimens, both males, were sacrificed to note the size and condition of the gonads, which were small, with no indication of gametogenesis, but neither of them contained a polystome. For a period of 3 weeks, pituitaries were dissected from frogs, *Rana pipiens*, and implanted in the dorsal lymph sacs of the 4 remaining animals. All of them were males. On dissection, a week later, their gonads were more than twice as large as those of the animals sacrificed at the beginning of the experiment and all contained spermatozoa. Three of the 4 were not infected, but the fourth, which had received 28 pituitaries, contained a polystome (Fig. 1). The worm is 4.3 mm long; 0.95 mm wide; and the suckers measure 0.29 mm in diameter. Morphologically, it agrees substantially with the

* Supplied by C. F. Kirk Co., Pharmaceutical and Biological Laboratories, New York, through the courtesy of A. L. Konwiser.

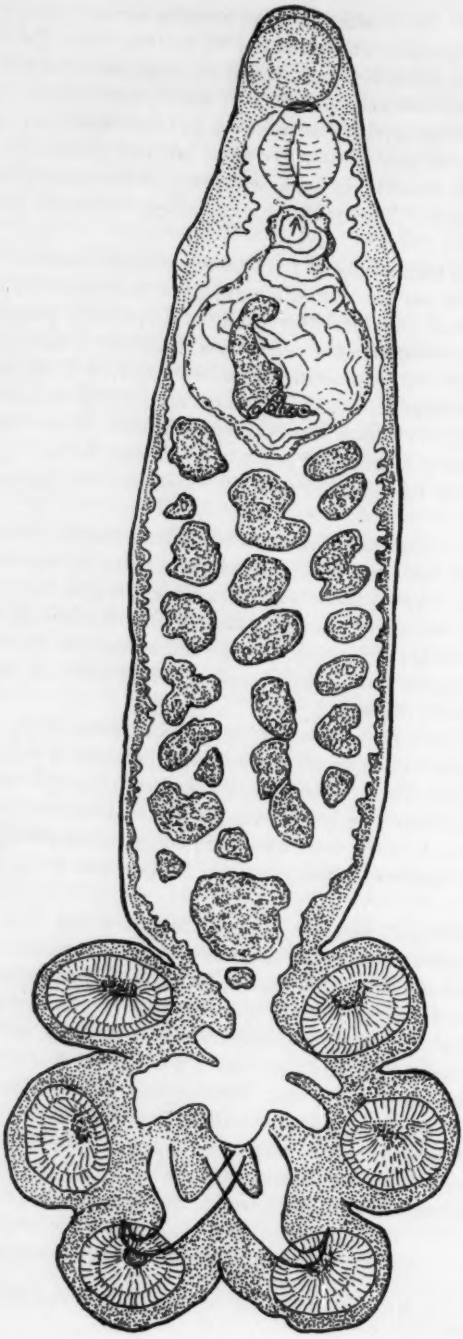

FIGURE 1. *P. stellai* from the urinary bladder of *Hyla septentrionalis*. The host, a half-grown individual, had received implants of 28 pituitaries from *R. pipiens* into the dorsal lymph sacs. In the parasite, the testes and ovary are functional but no eggs are present in the uterus.

description of *Polystoma stellai* as given by Vigeuras (1955) and is referred to that species. It is about one-half the size of the specimen described by Vigueras, but the testes are filled with spermatozoa and the ovary is functional, with ova in the oviduct.

The present report has been withheld in the hope that additional data could be obtained by repetition of the experiment. However, so far, no juvenile specimens of *H. septentrionalis* have been available and publication of the present account may lead other workers, in areas where the species is endemic, to repeat the work and further test the effect of pituitary implants or injection of gonadotropic preparations on the reproductive organs of *H. septentrionalis* and its polystome parasites.

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## RESEARCH NOTE

### PARASITES RECOVERED FROM SIX SPECIES OF UTAH LIZARDS

As a portion of the study of the helminths inhabiting the native fauna of Utah, the intestinal tracts of 47 lizards, belonging to 6 species were examined. The lizards were collected from desert valleys and low mountains of the Great Salt Lake Desert of Western Utah. Specimens of all species yielded helminths with the exception of the brown lizard, *Uta stansburiana stansburiana* Baird and Girard.

The desert horned lizard, *Phrynosoma platyrhinos* Girard, was found to harbor 4 species of parasites, and 15 of the 16 specimens examined had 1 to 4 species each. Helminths recovered were: CESTODA, *Oochoristica phrynosomatis* Harwood 1932; NEMATODA, *Skrjabinoptera phrynosoma* Ortlepp in the stomach, *Pharyngodon* sp. in the rectum, and a species of *Monhysterides* in the ileocecal region.

Two species of *Crotaphytus* are present in the region. Three of the 4 specimens of the western collared lizard, *C. collaris baileyi* Stejneger, were infected in the stomachs with *Physaloptera obtusissima* Molin 1860. Both the collared lizard and the leopard lizard, *C. wislizenii* Baird and Girard, possessed in the ileocecal region the same species of *Monhysterides* previously recovered from the desert horned lizard.

All 7 specimens of the Great Basin fence lizard, *Sceloporus occidentalis longipes* Bell, harbored numerous specimens of *Physaloptera retusa* Rudolphi, 1819, in the stomachs. Five of the 7 specimens dissected of the Great Basin whiptail lizard, *Cnemidophorus tigris tigris*, contained the cestode species *Oochoristca bivitellobata* Loewen, 1941, the round worm species *Pharyngodon werneri* Harwood, 1931, in the ileocecal region, and a specimen of the species *Angusticaecum*.

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# STUDIES ON THE HELMINTH FAUNA OF ALASKA. XXXIV. THE PARASITES OF WOLVES, *CANIS LUPUS* L.

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It is generally recognized that canine animals comprise an important reservoir of parasites and diseases transmissible to man. These animals, including the domestic dog, have unusual economic importance in arctic and subarctic North America, particularly for the Eskimos and Indians living in more remote regions. These aboriginal peoples continually have close association with canine animals and would appear to be quite vulnerable to infection from this source. That this is true is already evident, although data on morbidity from animal-borne diseases are far from complete. At the present time, at least in Alaska, these people have little ability to lessen the degree of exposure to zoonotic diseases, for poverty, ignorance of the problems, and adverse climatic conditions combine in making it difficult to effect sanitary practices necessary to prevent such exposures.

The investigation of parasites and diseases of canine animals in Alaska was initiated by Rausch in January, 1949. Many canids, representing 5 species, have been autopsied over the 10-year period, and it is believed that these surveys are now sufficiently complete to permit an evaluation of the potential importance of the helminths to human health. The findings for each canine species will be reported separately. It is the purpose of this paper to present observations on the helminths of wolves, *Canis lupus* L., with a review of pertinent literature.

## MATERIALS AND METHODS

Most of the wolves available for this work were obtained as skinned carcasses of animals shot or trapped by Eskimos and Indians in interior Alaska. These were labelled by the hunters, as required, and stored frozen until they could be transported to the laboratory. By this means, we benefited from the efforts of many hunters and secured more wolves than would have been possible in any other way. Also, because wolves in Alaska are decreasing in numbers as a result of general persecution, we desired to avoid killing any for our purposes since material was otherwise available.

A few animals were autopsied in the field during the warmer months, but the majority was examined in the laboratory. The organs were opened separately and the helminths were isolated in water. Since the wolves could not be examined immediately after death, many of the cestodes collected were in poor condition and not satisfactory for detailed study. Cestodes and trematodes were stained in Semichon's acetic carmine, and permanent mounts were prepared. The rostellar hooks of the *Taenia* spp. were removed and mounted separately, making possible more detailed study. The species of *Taenia* were tentatively separated after making enlarged projections of the hooks by means of a prism attached to a microscope, and comparing them directly with the outlines of the hooks of known species. This method permitted a rapid preliminary sorting of the large number of cestodes collected. Final determinations were based upon all characters of diagnostic value. The nematodes were cleared in lactophenol and studied unmounted.

The helminths considered herein were obtained from 200 wolves, the majority of which had been killed during the period November 1 through April 15. No animals younger than 6–7 months were examined. The localities from which these wolves were taken are shown in Figure 1.

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## RESULTS

### *1. CESTODA*

### Genus *Taenia* Linnaeus, 1758

Species of *Taenia* were recorded from 182 wolves (91% of the total). However, in view of the poor state of preservation of much of the material, the cestodes from only 78 animals were identified to species. Among the latter, 2 species occurred in 26 wolves, and 3 species were found in 13. The maximum number of strobilae recorded from a single animal was 66.

*Taenia hydatigena* Pallas, 1776

*T. hydatigena* was identified in 56 (72%) of 78 wolves. It has been recorded from this host in other North American localities by Erickson (1944) (Minnesota), and by Cowan (1946; 1947) (Vancouver Island and the Rocky Mountain parks of Canada). In Eurasia, Morozov (1951) found *T. hydatigena* in 12 of 20 wolves from the Mordvinian National Reserve, in the Middle Volga region; Petrov and Potekhina (1953) observed it in 1 of 2 animals examined in Tadzhikistan; Bondareva (1955) found the species twice among 12 animals autopsied in Kazakhstan; Rodonaia (1956) reported it from wolves in the Ladogan National Reserve, in the Leningrad area.

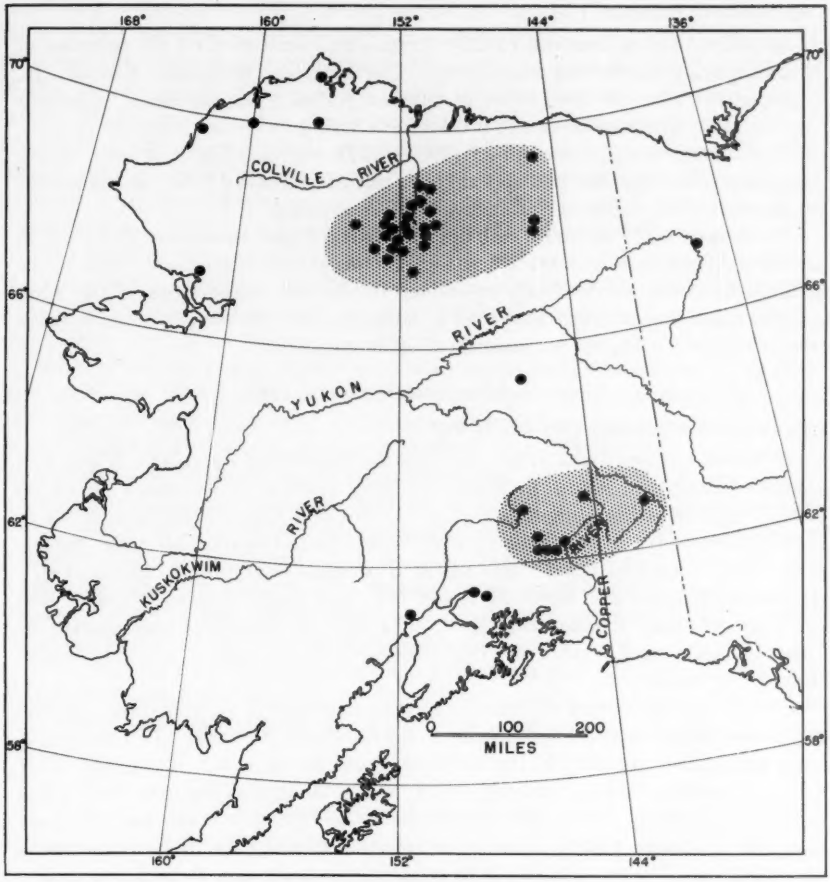
Wolves become infected through feeding upon deer, of which various species serve as the intermediate host of *T. hydatigena*. In Alaska, we have found the cysticerci in moose, *Alces alces gigas* Miller, and in caribou, *Rangifer tarandus stonei* Allen, wherever we have been able to examine such animals. The larval cestodes are commonly seen in the liver of the host, where they are often encysted in the hepatic surface just beneath the capsule. We have recorded a maximum number of 51 cysticerci from the liver of an old moose killed near Anchorage.

The larval *T. hydatigena* was first recorded from North American moose by Hall (1919), who reported it also in woodland caribou, *Rangifer caribou* (Gmelin), and in black-tailed deer, *Odocoileus hemionus* (Raffinesque). Ritcey and Edwards (1958) found it in 84% of 32 moose examined in British Columbia. Fenstermacher and Jellison (1933) reported an infected moose in Minnesota. Choquette *et al.* (1957) found this larval cestode to be the most common helminth in reindeer in the region of Aklavik, N. W. T. Gregson (1937) recorded it from a black-tailed deer in British Columbia, and Olsen and Fenstermacher (1943) found it in a white-tailed deer, *O. virginianus* (Zimmermann), in Minnesota. Cowan (1951) reported the cysticerci from several species of wild ruminants in British Columbia and Alberta. The Canadian records have been reviewed by Sweatman and Plummer (1957), who also made a detailed investigation of the larval development of this cestode. Machul'skii (1950) observed the cysticerci in 2 moose killed in Buriat-Mongolia.

*Taenia krabbei* Moniez, 1879

This cestode was found in 48 (61%) of 78 wolves. There appears to be no previous North American record from this host-species, but Morozov (1951) reported *T. krabbei* from 4 of 20 wolves examined in the Mordvinian National Reserve, in the western USSR.

The larval *T. krabbei* is a common parasite of caribou. Since these cysticerci are relatively small, and occur in the musculature of the host, it is difficult to determine




EXPLANATION OF FIGURE

FIGURE 1. Map of Alaska showing localities from which wolves were examined. The shaded areas indicate the central Brooks Range (to the north) and the Copper River drainage.

the infection rate accurately. We have observed infected caribou most frequently in the central Brooks Range region of northern Alaska, but they are often seen also in the Nelchina region of southern Alaska. We have not been able to examine any moose from regions where wolves are numerous, but this parasite is rare in moose in southern Alaska. Peterson (1955) reviewed records from moose and caribou in Canada, and Choquette *et al.* (1957) found 12.8% infected of 1664 reindeer examined over a 3-year period at Aklavik. Erickson and Highby (1942) reported these cysticerci from a woodland caribou in Saskatchewan. It is known to occur in reindeer in the Soviet Union (Skriabin, 1931), and in deer of other species in western Eurasia.

*Taenia multiceps* Leske, 1780

As pointed out by Cameron (1926), there is no justification for the retention of *Multiceps* as a genus distinct from *Taenia*. Our material is designated *T. multiceps*, in accordance with the conclusions of Clapham (1942), but the name *T. serialis* Gervais, 1847, would be applicable if Clapham's findings were not accepted.

*T. multiceps* was identified in 23 (29%) of 78 wolves. The only other North American record from this host appears to be that of Erickson (1944), in Minnesota. Bondareva (1955) reported it from a wolf in Kazakhstan.

We have collected the larval cestodes from hares, *Lepus americanus* Erxleben, in southern Alaska during years when these animals were numerous. The hooks obtained from the larvae closely resembled in size and shape those of the adult cestodes taken from wolves. Larvae of *T. multiceps* have not been observed in hares from northern Alaska, nor in mammals of other species.

Genus *Echinococcus* Rudolphi, 1810

*Echinococcus granulosus* (Batsch, 1786)

*E. granulosus* was found in 60 (30%) of the 200 wolves autopsied. It was first reported from North American wolves by Riley (1933), in Minnesota; Erickson (1944) reported it from the same region. DeVos and Allin (1949) considered *E. granulosus* to be the most common cestode in wolves in Ontario; here also, Sweatman (1952) found 36 (62%) infected of 58 animals examined. Cowan (1947) reported an infected wolf from the Rocky Mountain parks of Canada and (1948) one from Alberta. *Echinococcus* sp. from a wolf in Macedonia was studied by Cameron (1926) and Bondareva (1955) recorded *E. granulosus* in 1 of 12 wolves autopsied in Kazakhstan.

Deer of various species serve as the intermediate host of *E. granulosus*, caribou and moose being the important species in boreal North America. The larval cestodes are found commonly in the lungs of moose in the lower Matanuska River valley, in southern Alaska (Rausch, 1952); this is an agricultural area from which wolves are generally absent, and dogs probably are the only important final host. Here, R. A. Rausch (1959) observed an infection rate of 63% in 16 moose which were all at least 7 years old. In 124 moose from the Anchorage-Palmer area, representing all age-classes, he found the rate of infection to be 20%. A maximum number of 18 cysts has been observed in the lungs of a single moose (Rausch, 1952).

Infected moose have been reported in Canada by Hadwen (1932) (Manitoba), Law and Kennedy (1933), deVos and Allin (1949), and Sweatman (1952) (Ontario). Harper *et al.* (1955) found 29 moose infected of 96 examined in Saskatchewan, and Ritcey and Edwards (1958) observed the larvae in 68% of the animals autopsied in British Columbia. The records of the larval *E. granulosus* from moose have been reviewed by Peterson (1955). Farther south, infected moose have been reported by Riley (1933; 1939), Fenstermacher and Jellison (1933), and Olsen and Fenstermacher (1942) (Minnesota). Sweatman (1952) cited a record from Isle Royale.

Our observations indicate that the rate of infection in caribou in Alaska is relatively low. Since 1949, Rausch has examined grossly the lungs of more than 200 caribou from the central Brooks Range without having found any infected. A single pulmonary cyst was observed in an old bull in the Nelchina area (Rausch, 1952). In the latter area during 1956–57, R. O. Skoog, U. S. Fish and Wildlife

Service, examined 67 caribou representing all age-classes; of these, only 4 animals, all at least 7 years old, were infected (personal communication). Banfield (1951) reported infected caribou from northern Manitoba and from the Northwest Territories, and Sweatman (1952) cited a record from the Keewatin District. Harper *et al.* (1955) found 3 caribou infected among 14 examined in Saskatchewan.

Hadwen and Palmer (1922) implied that Alaskan reindeer are sometimes infected, although specific records were not given. We have not found this parasite in reindeer on Nunivak Island, nor have we any information about its occurrence in the smaller herds on the mainland of Alaska. Over a 3-year period, Choquette *et al.* (1957) noted an infection rate of 9.5% for 1664 deer examined at Aklavik, N. W. T. *E. granulosus* is a common parasite of reindeer in northern Scandinavia (cf. Söderhjelm, 1945; 1946), and is known also from reindeer in the Soviet Union (cf. Skriabin, 1931).

In North America, deer of other species are sometimes found infected. We have one record from a black-tailed deer from Baranof Island, southeastern Alaska, where wolves are not known to occur. The same species has been found infected on Vancouver Island and in Alberta by Cowan (1946). Riley (1939) found this cestode in 1 of 58 white-tailed deer examined in Minnesota, and Olsen and Fenstermacher (1943) also reported it from this host-species in the same area. Harper *et al.* (1955) recorded 2 infected white-tailed deer in Saskatchewan, and Sweatman (1952) found a single animal infected among 353 examined in Ontario. Green (1949) examined the lungs of 1,073 wapiti, *Cervus canadensis* (Erxleben), over a 4-year period in Alberta, where he observed the larval *E. granulosus* in 57 animals (5%), most of which were over 2 years of age.

We also have identified this parasite in a mountain goat, *Oreamnos americanus* (Blainville), which was shot near the head of the Lynn Canal, southeastern Alaska. This animal had 2 pulmonary cysts, of which the larger measured 70 mm. in diameter.

### *2. TREMATODA*

### Genus *Alaria* Schrank, 1788

*Alaria canis* LaRue and Fallis, 1936

*A. canis* was the only species of trematode recorded from Alaskan wolves; it was found in 8 (4%) of the 200 animals autopsied. The occurrence of this parasite in wolves has been mentioned by Babero and Rausch (1952). The only other published record from this host appears to be that of Pearson (1956), who found it in 10 of 34 wolves autopsied in Ontario.

According to the findings of Pearson (1956), it would be possible for wolves to become infected through eating frogs containing the mesocercariae of *A. canis*. Possibly, also, a paratenic host, probably a rodent, is involved. One species of frog, *Rana sylvatica cantabrigensis* Baird, is widely distributed in Alaska, with its range restricted essentially to the taiga zone. Only 1 (0.6%) of 162 wolves from the Brooks Range was infected. By comparison, 7 (25%) of 27 animals from the Copper River drainage in southern Alaska, where frogs are numerous, were infected (Fig. 1).

### *3. NEMATODA*

### Genus *Trichinella* Railliet, 1895

*Trichinella spiralis* (Owen, 1835)

Larval nematodes of the genus *Trichinella,* identified as *T. spiralis,* were found in 51 (33%) of the 154 wolves examined for this parasite. The occurrence of this nematode in wolves has been discussed elsewhere (Rausch *et al.,* 1956).

Genus *Uncinaria* Froelich, 1789

*Uncinaria stenocephala* (Railliet, 1884)

This hookworm was collected from 14 (7%) of the 200 wolves examined. *U. stenocephala* was first recorded from North American wolves by Erickson (1944), who found it to be rather common in these animals in northern Minnesota. Rodonaia (1956) reported it from wolves in the Ladogan National Reserve.

Only 7 (4%) of the 162 wolves from the central Brooks Range were infected, compared with 7 (25%) of 27 animals from the Copper River drainage. Although the latter series is too small to permit any definite conclusions, the difference in infection rates between the tundra and taiga zones appears significant.

Genus *Toxascaris* Leiper, 1907

*Toxascaris leonina* (von Linstow, 1902)

*T. leonina* was a very common parasite, occurring in 144 (72%) of the wolves. There appears to be no previous record from this host in North America. Morozov (1951) found it in 4 of 20 animals examined in the Mordvinian National Reserve, and Petrov and Potekhina (1953) reported it from 2 wolves in Tadzhikistan.

Of 162 wolves from the central Brooks Range, 136 (84%) were infected, as compared with only 6 (22%) of 27 animals from the Copper River drainage. It was not unusual to find 50 to 60 of these ascarids in an individual wolf, and a maximum number of 175 worms was noted in an animal from the Brooks Range.

## DISCUSSION

Eight species of helminths were recorded from Alaskan wolves in the course of the present survey. Of these, 6 occurred commonly and 2 were found in less than 10% of the 200 animals considered. The number of species in wolves is small compared with what we have observed in other Alaskan canids (e.g., dog and arctic fox), perhaps indicating that wolves have much more restricted feeding habits.

Wolves are found throughout the taiga and tundra zones of Alaska, excepting those regions where wild ruminants are absent, or where they have been driven out by man. During the colder months, wolves follow the migrating herds of caribou, upon which they depend. The most diversified diet is had during the spring and early summer, when they remain in the vicinity of the den; during this time, any animals that can be captured are used as food. Carrion is sometimes consumed, but it usually comprises a small proportion of their diet.

Caribou and moose serve as the intermediate host for *Taenia hydatigena, T. krabbei,* and *Echinococcus granulosus.* The larvae of the first 2 species occur so commonly in caribou that it would seem impossible that any wolf could long remain uninfected. There is some evidence that wolves kill a disproportionally large number of aged caribou; this would increase the probability of their ingesting the larvae of the aforementioned species of cestodes, since the infection rate is known to increase with age in these deer. Aside from the fact that wolves often travel in close association with herds of caribou, and deposit their feces where caribou feed, noth-

ing is known about the infection of the intermediate host of these cestodes. Opportunities for their becoming infected would seem to be much reduced by the fact that they rarely feed long in one area. Seasonal differences in feeding behavior may be important. Söderhjelm (1946) and others have pointed out that reindeer seek out and eat urine-soaked snow, but it has not been reported whether they also may eat feces of canine animals.

*T. multiceps* occurs in larval form in hares, but hares do not generally comprise an important part of the diet of wolves. It is conceivable that in years when hares are especially numerous they may become an important food of wolves, since abundance and availability of a prey-species seem to determine the degree to which it is preyed upon. At times of high population density of hares, many may be utilized when the young wolves are in the den. It has not been possible to obtain information which would permit any correlation between the abundance of hares and the prevalence of *T. multiceps* in wolves, although the infected animals reported herein were killed during years immediately preceding a peak hare population in the central Brooks Range. If wolves in Alaska become infected only from hares, the prevalence rate of *T. multiceps* would be expected to decrease during times of hare scarcity.

Both *Trichinella spiralis* and *Alaria canis* are acquired by wolves from animals other than the aforementioned cervids. The specific origin of *Trichinella* larvae is unknown, although there are several possible sources (Rausch *et al.*, 1956). In the case of *A. canis,* wolves may ingest mesocercariae in frogs, or they may become infected through some paratenic host. It appears doubtful that microtine rodents eat frogs or their larvae, although such rodents may be more omnivorous than is now recognized.

*Toxascaris leonina* can develop directly in the final host when infective eggs are ingested by a susceptible animal, or infection may be acquired through the eating of rodents containing the encysted larvae (Sprent, 1954). During the spring and early summer, eggs eliminated by the adult wolves in the vicinity of the dens would have time to become infective to the pups before the latter left the den area. The situation appears similar in the case of *Uncinaria stenocephala.* For both species, the adult wolves probably serve as reservoir hosts from which the young animals indirectly become infected. There is no evidence that the eggs of either species can survive the low winter temperatures characteristic of the higher latitudes.

Besides those species recorded from Alaskan wolves, several helminths, to all appearances characteristic of a more southern fauna, have been reported in wolves at lower latitudes in both North America and Eurasia. Certain others, such as *Diphyllobothrium* spp., can be expected; the presence of *Diphyllobothrium* sp. in a wolf in Ontario (deVos and Allin, 1949) may not be unusual, since these animals must on occasion capture and eat fishes containing plerocercoids of *Diphyllobothrium.* Another species, *Echinococcus multilocularis* Leuckart, 1863, has been grown experimentally in a wolf (Rausch, 1959); its natural occurrence in this host in Alaska would depend upon the utilization of microtine rodents in certain regions. A few species of helminths, e.g., *Alaria alata* (Goeze, 1782), are as far as is known distributionally restricted to Eurasia.

The parasites of Alaskan wolves recognized as being pathogenic to man are *Echinococcus granulosus* and *Trichinella spiralis,* although it is improbable that either is often transmitted directly from wolves to man. Infection by the former

may occur in hunters and trappers through their accidental ingestion of *Echinococcus* eggs after handling wolf carcasses, but the casual infection from this source seems insignificant upon realization that the same hunters may live in close association with infected dogs.


## SUMMARY

Two hundred wolves, *Canis lupus,* L., have been examined for parasitic helminths over a 10-year period in Alaska. Most of these animals (162) were killed in the Brooks Range region of arctic Alaska.

Eight species of helminths have been identified: *Taenia hydatigena* Pallas, 1776; *T. krabbei* Moniez, 1879; *T. multiceps* Leske, 1780; *Echinococcus granulosus* (Batsch, 1786); *Alaria canis* LaRue and Fallis, 1936; *Trichinella spiralis* (Owen, 1835); *Uncinaria stenocephala* (Railliet, 1884); *Toxascaris leonina* (von Linstow, 1902).

Observations on the occurrence, distribution, and natural history of these helminths have been presented, with a review of pertinent literature.

Two species, *Echinococcus granulosus* and *Trichinella spiralis,* are pathogenic for man, but it is concluded that wolves have little importance as reservoir hosts from which man may be infected.



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Personnel of the U. S. Fish and Wildlife Service made available wolf carcasses from southern Alaska, and others were obtained through the assistance of the Arctic Research Laboratory, Office of Naval Research, at Point Barrow. Dr. J. F. A. Sprent, University of Queensland Veterinary School, Brisbane, Australia, made detailed studies of a series of *Toxascaris leonina* from Alaskan canids. We wish to express our appreciation for this assistance.


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(*continued from p. 384*)

appreciably the carapace of the crab. They feed mostly, perhaps exclusively, at the soft articulations on the venter. This leech has no hard jaws or teeth capable of cutting even soft tissues. It feeds on the blood and juices of the host by suction and the anticoagulant action of its salivary secretion. While I have not tested the acidity of the latter it certainly is not equal to dissolving the hard calcareous shell." Moore (1946, Notul. nat. Acad. Philad., No. **184:** 1–12) discusses the anatomy and systematic position of *M. lugubris.* He states that although *M. lugubris* is seldom mentioned in the literature, ". . . it is very common on the edible blue crab, now known as *Callinectes sapidus,* in the brackish coastal waters of New Jersey, Chesapeake Bay and North Carolina and probably wherever the blue crab occurs in both brackish and fresh-water." One of us (F. S.) has observed at least 500 blue crabs from the brackish waters of Lake Pontchartrain, Louisiana, and the other of us (R. F. H.) has observed specimens of *C. sapidus* over a period of 10 years in various brackish-water habitats on both coasts of Florida, but we have not observed leeches on any of these crabs. However, Dr. Gordon Gunter, Director, Gulf Coast Research Laboratory, Ocean Springs, Mississippi (personal communication) reports observing a leech attached to *C. sapidus* from either the St. Lucie estuary or the Calcosahatchee estuary during the early part of 1958. Meyer and Barden (1955, Wasmann J. Biol. **13:** 297–311) record *Myzobdella lugubris* from Texas and Louisiana waters. Also, they give a record of this worm being attached to the prawn (*Palaemonetes vulgaris* Say) and oysters.

Leidy (1852, Proc. Acad. nat. Sci. Philad., 1851–3, p. 243) created the genus *Myzobdella* for this species. He stated that it was "parasitic upon the common edible crab, *Lupa dicantha,* M. Edw.; usually found attached about the base of the limbs." Rathbun (1930, Bull. U. S. nat. Mus., No. **152:** p. 99) considers the name *Lupa dicantha* M. Edw., to be a synonym for the blue crab, *Callinectes sapidus* Rathbun.

Our report of *Myzobdella lugubris* Leidy appears to be the first record of the leech in Florida waters. This report therefore extends the southerly geographical distribution of *M. lugubris.* In addition, this is evidently the first time *M. lugubris* has been indicated as a possible factor in mortality of the blue crab.

Acknowledgments are here extended to Dr. J. Percy Moore for his kindness in identifying the leech for us and for his comments on the feeding habits of *M. lugubris.*—Robert F. Hutton and Franklin Sogandares-Bernal, *Florida State Board of Conservation Marine Laboratory,* Maritime Base, Bayboro Harbor, St. Petersburg, Florida.* (* Contribution No. 12)

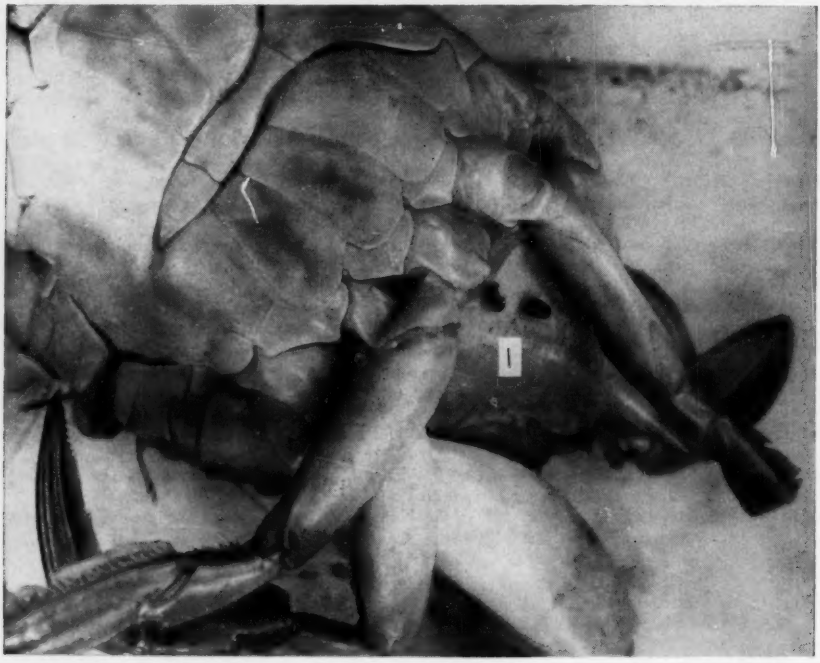

Figure 1. Two specimens of the leech, *Myzobdella lugubris* Leidy, attached to the carapace of the blue crab, *Callinectes sapidus* Rathbun.

(*continued on p. 430*)

# THE IMMATURE STAGES OF THE GENUS *HOPLOPLEURA* (ANOPLURA: HOPLOPLEURIDAE) IN NORTH AMERICA, WITH DESCRIPTIONS OF TWO NEW SPECIES*

EDWIN F. COOK AND JAMES R. BEER

The immature stages of the genus *Hoplopleura* are but little known. Of the known North American species only a few have been described or illustrated. Ferris (1921, 1951) illustrated 1 stage of *H. acanthopus* (Burm.) and *H. arboricola* K. and F., and Pratt and Karp (1953) illustrated all stages of *H. oenomydis* Ferris. In addition to this, a brief description of 1 stage of *H. hirsuta* Ferris (Ferris, 1921) and *H. oryzomydis* P. and L. was made by Pratt and Lane (1951).

In the course of collecting specimens for quantitative studies on louse populations, numerous immature stages have come to hand representing most of the described North American species. All stages of North American species have been collected by us with the exception of *H. oenomydis, H. hirsuta* and *H. oryzomydis*. Specimens representing *H. oenomydis* have been made available to us through the courtesy of Dr. Harry Pratt of the C.D.C. in Atlanta. Skins of *Oryzomys palustris* and *Sigmodon hispidus* from which *H. hirsuta* and *H. oryzomydis* were recovered were made available to us by Dr. Norman Negus of Tulane University.

The adult stages have all been carefully described and precisely illustrated by Ferris (1921, 1951), and also to some extent by Pratt and Lane (1951). Keys to the adult stages are also available in Ferris (1951) and Pratt and Lane (1951). In the present paper, in addition to the key and descriptions of all immature stages, 2 new species are described, 1 of which is readily distinguished in both adult and nymphal stages and another which can be distinguished only in the immature stages. Characters are also considered which will more readily permit the separation of *H. arboricola* from *H. erratica* in adult stages.

The several nymphal instars are easily recognized, since in any long series of specimens at least a few nymphs will be found on the point of molting. Succeeding instars are at this time visible within the nymphal skin of the preceding stage. It is apparent in this material that there are 3 nymphal stages.

It is evident that the North American species of the genus are separable into 3 groups. Those nymphs found on chipmunks and tree squirrels (*H. arboricola, H. erratica,* and *H. sciuricola*) have 5 visible pairs of minute abdominal spiracles. Those found on cricetid rodents have no such spiracles visible in any immature stages. The 1 species found on the flying squirrel (*H. trispinosa*) lacks abdominal spiracles as a nymph, but has the anal lobes apically setose in all stages, a condition not evident in the other 2 groups. The lice of chipmunks and tree squirrels also have conspicuous dorsal and ventral abdominal setae in addition to the marginal

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setae in the 2nd and 3rd instars. These are not present at all or are minute in the early stages of the other 2 groups.

Two complexes requiring further investigation have been revealed here. One of these complexes is found on *Peromyscus* spp. and the related *Onychomys* spp. The 2 new species herein described are from this complex. These new species are very close to *H. hesperomydis*. The other complex is found on *Eutamias* spp. Considerable variation is evident in the material we have had available from various species of *Eutamias* from different areas in North America, but our samples are not large enough nor from enough *Eutamias* species to permit us to recognize any new species here. This will be considered further in the descriptions of *H. arboricola*.

No further speculation on the classification of this widely distributed genus is justified on the basis of the limited North American fauna, but examination of immatures of other species should clarify the relationships in this group.

All North American species of the genus *Hoplopleura* can be recognized in the 3 nymphal stages by the presence of small unpigmented spines variously distributed on the ventral surface of the head capsule, the first antennal segment, and the coxae.

The illustrations of immature specimens that accompany the following key and descriptions are semi-diagrammatic.

KEY TO THE NYMPHS OF *Hoplopleura* OF NORTH AMERICA

1. With 2 or more major posterior abdominal setae ..... 3
1a. With no major posterior abdominal setae ..... 2
2. With several pairs of minute, ventral abdominal setae; without seta pair X (Fig. 15) 2nd instar, *oenomydis*
2a. With only 1 pair of minute, ventral abdominal setae; with seta group X (Fig. 25) 3rd instar, *oenomydis*
3. With only 2 major posterior abdominal setae ..... 4
3a. With 4 or more major posterior abdominal setae ..... 5
4. Abdomen with a double row of 6 sclerotized plates dorsally (Fig. 5) 1st instar, *oenomydis*
4a. With no dorsal plates (Fig. 20) ..... 2nd instar, *oryzomydis*
5. Abdominal spiracles present (Figs. 13 and 14; Figs. 23 and 24; Figs. 29, 30, 31) .... 6
5a. Abdominal spiracles absent ..... 14
6. Abdomen with 4 or more pairs of dorsal setae near midline of posterior portion (Fig. 14; Fig. 23) ..... 7
6a. Abdomen with no more than 1 pair of dorsal setae near midline of posterior portion (Fig. 13; Fig. 24) ..... 12
7. Abdomen with only 4 pairs of dorsal setae near midline of posterior portion (Fig. 14) 11
7a. Abdomen with 9 pairs of dorsal setae extending from anterior part of abdomen to apex (Fig. 23; Figs. 30 and 31) ..... 8
8. With no trace of paratergal sclerites ..... 9
8a. With paratergal sclerites (Figs. 30 and 31) ..... 10
9. From *Eutamias* (Fig. 23) ..... 2nd instar, *arboricola*
9a. From *Tamias* ..... 2nd instar, *erratica*
10. Paratergal lobes without setae; from *Tamias* (Fig. 30) ..... 3rd instar, *erratica*
10a. Paratergal lobes with setae; from *Eutamias* (Fig. 31) ..... 3rd instar, *arboricola*
11. From *Eutamias* (Fig. 14) ..... 1st instar, *arboricola*
11a. From *Tamias* ..... 1st instar, *erratica*
12. With 4 major setae at posterior end of abdomen (Fig. 13) ..... 1st instar, *sciuricola*
12a. With 12 major setae at posterior end of abdomen ..... 13
13. One pair of anterior abdominal setae (Fig. 24) ..... 2nd instar, *sciuricola*
13a. Three pairs of anterior abdominal setae (Fig. 29) ..... 3rd instar, *sciuricola*
14 Posteriormost abdominal lobe with 4 to 6 small setae (Figs. 12; 22) ..... 15
14a. No small setae on posterior abdominal lobe ..... 17
15. With 4 major setae at posterior end of abdomen ..... 16
15a. With 8 major setae at posterior end of abdomen (Fig. 28) ..... 3rd instar, *trispinosa*
16. With a single pair of small lateral setae on thoracic tergum in addition to principal setae of thorax; no minute setae near midline of dorsum of head (Fig. 12) 1st instar, *trispinosa*

16a. With 2 pairs of small lateral setae on thoracic tergum in addition to principal setae of thorax; 2 pairs of minute setae ("X") near midline of dorsum of head (Fig. 22) 2nd instar, *trispinosa*

17. With only 4 major setae at posterior end of abdomen (Figs. 10 and 11) ..... 18

17a. With 4 or more major abdominal setae plus 2 minor setae adjacent to ventral majors (as Figs. 5, 6, 7, 8) ..... 19

18. Principal posterior dorsal head setae long, reaching beyond principal thoracic dorsal; principal thoracic dorsal nearly attaining end of abdomen (Fig. 11). 1st instar, *oryzomydis*

18a. Principal posterior dorsal head setae short, not attaining base of principal dorsal thoracic setae (Fig. 10) ..... 1st instar, *hirsuta*

19. Abdomen with only 4 major posterior abdominal setae plus 2 minor setae adjacent to ventral majors ..... 20

19a. Abdomen with more than 4 major posterior abdominal setae ..... 23

20. Principal anterior dorsal head setae, principal posterior dorsal head setae, and principal dorsal thoracic setae of same size, stout; no small setae adjacent to posterior dorsal head setae (Figs. 6, 7, 8) ..... 21

20a. Principal anterior dorsal head setae much smaller than principal posterior dorsals; principal posterior dorsal with a small seta mesad of base (Fig. 9) . 1st instar, *acanthopus*

21. Abdomen with transverse grooves marking off 8 abdominal segments; minute setae on some segments (Figs. 7 and 8) ..... 22

21a. No segmentation apparent on abdomen; minute abdominal setae rare or absent (Fig. 6) 1st instar, *hesperomydis*

22. Terminal abdominal segment prolonged posteriorly; most sternal segments with a pair of minute setae (Fig. 8) ..... 1st instar, *ferrisi*

22a. Terminal abdominal segment not particularly prolonged, only 2 pairs of minute abdominal setae present (Fig. 7) ..... 1st instar, *onychomydis*

23. Abdomen with 6 major posterior abdominals ..... 24

23a. Abdomen with more than 6 major posterior abdominals ..... 27

24. Anterior and posterior principal dorsal setae of head and major thoracic dorsal of the same size, stout; no small setae adjacent to base of posterior dorsal head setae (Figs. 16, 17, 18) ..... 25

24a. Anterior dorsal head setae much smaller than major posterior dorsal head setae; base of posterior dorsal head setae with a small seta adjacent mesally (Fig. 26) 3rd instar, *oryzomydis*

25 Terminal abdominal segment prolonged (Fig. 18) ..... 2nd and 3rd instar, *ferrisi*

25a. Terminal abdominal segment not especially prolonged ..... 26

26. Several pairs of minute setae on ventral side of abdomen (Fig. 17) from *Onychomys* 2nd and 3rd instar, *onychomydis*

26a. Only 1 or 2 pairs of minute setae at most on ventral side of abdomen (Fig. 16) from some *Peromyscus* spp. ..... 2nd and 3rd instar, *hesperomydis*

27. No small setae at posterior end of abdomen ..... 28

27a. Small setae both dorsally and ventrally in addition to 8 major setae at posterior end of abdomen (Fig. 19) ..... 2nd and 3rd instar, *acanthopus*

28. 8 major posterior abdominal setae (Fig. 21) ..... 2nd instar, *hirsuta*

28a. 12 major posterior abdominal setae (Fig. 27) ..... 3rd instar, *hirsuta*

*Hoplopleura onychomydis* n. sp.

*Females:* (Fig. 4.) Total length, 0.72–1.15 mm. (M, 0.94.) (Holotype, 0.95.) Head and thorax identical with those of *H. hesperomydis* except posterior process of sternal plate blunt and not acute (Fig. 2). Paratergal plates (Fig. 3) similar to those of *H. hesperomydis,* except lateral lobes more deeply emarginate and setae in cleft of plates 4, 5 and 6 both readily visible under 20× magnification. In *H. hesperomydis,* the dorsal seta of each pair nearly invisible. Tergal and sternal abdominal plates and chaetotaxy identical with *H. hesperomydis.*

*Males:* (Fig. 1.) Total length 0.74–0.82 mm. (M, 0.79.) (allotype, 0.77.) Sternal plate of thorax as in female; paratergal plates like those of female except for 7 in which the lateral processes are shorter and not acute. Chaetotaxy of abdomen like that of male *H. hesperomydis.* Genitalia like those of *H. hesperomydis* except apex of parameres not enlarged.

*Nymph 1:* (Fig. 7.) Total length 0.28–0.39 mm. Very similar to nymph of *H. hesperomydis* except principal head setae slightly smaller. Anterior and posterior principal head setae of same size; thoracic principal smaller than principal head setae. Abdomen clearly segmented, with a single pair of minute setae dorsally posterior to major abdominal setae and 2 pairs of minute setae ventrally on apparent segments 5 and 6. Each major ventral seta of abdomen with

adjacent small seta ventrad of base. Last abdominal segment not prolonged posteriorly. Cuticle of abdomen appears scaly, of a light brown color. Four major abdominal setae.

*Nymph 2:* (Fig. 17.) Total length 0.33–0.45 mm. Similar to *H. hesperomydis* except for 2 to 4 pairs of minute setae on ventral side of abdomen; 2 pairs of small setae on dorsum of thorax in addition to dorsal principal. Dorsal principal of thorax conspicuously smaller than principal head setae; 6 major abdominal setae; cuticle like that of nymph 1. The largest specimens here were in the process of molting into nymph 3.

*Nymph 3:* Total length 0.44–0.49 mm. This stage is identical with nymph 2 except for slightly larger size. The sizes cited here may be too small since only 7 third instars were present in the collection and none of them were molting.

*Specimens examined:* Holotype: female, *ex Onychomys torridus,* Portal, Arizona, August 13, 1957. Allotype: male, *ex. Onychomys torridus,* Portal, Arizona, July 27, 1957. Paratypes: 13 males, 11 females, 24 first instars, 21 second instars, and 7 third instars. All collected from 9 *Onychomys torridus,* Portal, Arizona, July 27 to August 15, 1957, James R. Beer and Robert Schwab. Type locality: Portal, Arizona. Type host: *Onychomys torridus.* Types in the University of Minnesota collection.

*Comments:* This species will key in Ferris (1951) to *H. hesperomydis.* It can be readily separated from that species by the blunt posterior process of the sternal plate in both the males and females. The immature stages can be separated by the characters given in the key.

It seems likely that the specimens recorded as *H. hesperomydis* by Ferris (1921 and 1951) from *Onychomys torridus* in California are also this species.

*Hoplopleura ferrisi* n. sp.

*Females:* As far as can be determined, the females are identical with those of *H. hesperomydis.*

*Males:* These are also identical with the males of *H. hesperomydis.*

*Nymph 1:* (Fig. 8.) Total length 0.31–0.35 mm. Similar to nymph 1 of *H. hesperomydis* except principal head setae slightly smaller and no other head setae are apparent. It differs also from *H. hesperomydis* in having 7 pairs of minute ventral abdominal setae and in having an extremely long, slender anal segment. This species differs from *H. onychomydis* in the larger number of ventral abdominal setae, in having no minute dorsal setae before the anal segment, and in having no dorsal head setae except for the dorsal principals. Four major abdominal setae present; cuticle of abdomen appearing scaly, light brown in color. Abdomen with intersegmental folds.

*Nymph 2:* (Fig. 18.) Total length 0.35–0.55 mm. Similar to nymph 2 of *H. hesperomydis* and *H. onychomydis.* Major abdominal setae very large; anal segment long and slender and, thus, conspicuously different from any other species in the genus. Six major abdominal setae. Cuticle like that of nymph 1.

*Nymph 3:* Essentially identical with nymph 2. Total length 0.68–0.75 mm.

*Specimens examined:* Holotype: 1st instar nymph from *Peromyscus boylei,* Portal, Arizona, August 5, 1957, collected by James R. Beer and Robert Schwab. Paratypes: 10 males, 17 females, 13 first instars, 33 second instars, 1 third instar. All from the same host specimen.

*Also examined:* 67 males, 89 females, 71 first instars, 128 second instars, and 35 third instars from 12 *Peromyscus nasutus,* Portal, Arizona, August 5 to August 9, 1957, by James R. Beer and Robert Schwab; 4 males, 6 females, 17 first instars, and 8 second instars, from 3 *Peromyscus eremicus,* Wilna, New Mexico, June 3, 1955, James R. Beer. Type locality: Portal, Arizona. Type host: *Peromyscus boylei.* Types in the University of Minnesota collections.

*Comments:* This species is most remarkable in that it can only be identified in the immature stages. It is very close to both *H. hesperomydis* and *H. onychomydis.* A few immature specimens of *H. hesperomydis* from *Peromyscus maniculatus* have been examined from Arizona and these are identical with the immatures from *P. maniculatus* in Minnesota, Oregon, and Wyoming that we have seen. This is not, therefore, a geographical variant of *H. hesperomydis.* It may well be that a careful analysis of the parasite populations of the various species of the genus *Peromyscus* will reveal even more new species (See further comments under *H. hesperomydis*).

This species is respectfully dedicated to the late Professor G. F. Ferris to whom the senior author owes a most profound debt of gratitude for guidance, assistance and inspiration both personal and scientific. Professor Ferris' exemplary work in this group has provided the great bulk of our knowledge of Anoplura.

## DESCRIPTION OF THE EARLY INSTARS OF KNOWN NORTH AMERICAN SPECIES

*Hoplopleura hesperomydis* Osborne

*Nymph 1:* (Fig. 6.) Total length, 0.30–0.37 mm. Anterior principal head setae larger than posterior. Principal thoracic setae similar in size to posterior principal head setae. Abdomen usually without paired minute setae except for those adjacent to major ventral abdominals; 4 major abdominal setae; no evidence of abdominal segmentation; cuticle all pale, not appearing scaly; Anal segment not prolonged posteriorly.

*Nymph 2:* (Fig. 16.) Total length, 0.34–0.47 mm. Anterior and posterior principal head setae and principal thoracic setae of same size. Anal segment not prolonged posteriorly; no minute setae on abdomen (rarely 1 pair anteriorly on venter) except for the ventral pair mesad of the posterior major abdominals; 6 major abdominals present.

*Nymph 3:* Total length 0.60–0.79 mm. Otherwise identical with nymph 2.

*Specimens examined:* Over 300 specimens from *Peromyscus maniculatus,* Basswood Lake and Rosemount, Minnesota; Beverly Beach State Park, Oregon; Douglas, Wyoming; and Valle, Arizona. Also from *Peromyscus leucopus,* Cedar Creek Forest, Anoka County, Minnesota.

*Comments:* This species together with the 2 preceding species (*H. ferrisi* and *H. onychomydis*) form a natural and closely related group characterized by the highly developed anterior principal head seta in all nymphs, and the presence of only 6 major posterior abdominal setae in the 2nd and 3rd instars. This species is readily separated from *ferrisi* and *onychomydis* in the 1st instars by the lack of any trace of abdominal segmentation and in 2nd instars by a lack of minute setae on the ventral side of the abdomen (occasional specimens have a single pair of such setae present). The adult can be distinguished from *H. onychomydis* but not from *H. ferrisi.* Records of this species on *Peromyscus boylei* (Ferris 1951) refer most likely to *H. ferrisi.* Records on *Onychomys torridus* probably refer to *H. onychomydis* while those from *Oryzomys* probably refer to *H. oryzomydis* Pratt and Lane.

### *Hoplopleura acanthopus* (Burmeister)

*Nymph 1:* (Fig. 9.) Total length 0.42–0.57 mm. Anterior principal head setae represented by the inner one of a pair of minute setae located anterior to clypeofrontal suture. Posterior principal head seta large, extending to middle of thorax. This seta with a minute seta located mesad of base. Thoracic dorsal principals as large as posterior principal setae of head. Four major abdominal setae, a minute seta located mesad of base of each ventral major abdominal seta; no evidence of abdominal segmentation; cuticle of abdomen not pigmented.

*Nymph 2:* (Fig. 19.) Total length 0.50–0.70 mm. Anterior principal head setae minute. Posterior principal nearly attaining posterior margin of head. Principal thoracic setae somewhat larger. Eight major abdominal setae. A pair of minute setae located dorsally, posterior to posterior major abdominals. Two pairs of minute setae anterior to and one pair posterior to posterior major abdominals ventrally; no evidence of abdominal segmentation, scales, or pigmentation on abdomen.

*Nymph 3:* Total length 0.64–0.80 mm. This stage is identical, except for size, with stage 2. It can only be positively identified when the adult structure is visible internally.

*Specimens examined:* 65 first instars, 32 second instars, and 7 third instars, from *Microtus pennsylvanicus,* Rosemount and St. Paul, Minnesota. All of these specimens were slide mounted. Several thousand specimens have been examined in alcohol.

*Comments:* This species is found on *Microtus pennsylvanicus* throughout its range and in small numbers on *Clethrionomys gapperi and Synaptomys cooperi* in Minnesota.

### *Hoplopleura oryzomydis* Pratt and Lane

*Nymph 1:* (Fig. 11.) Total length 0.36–0.41 mm. Anterior principal head setae represented by the inner of a minute pair just anterior to clypeofrontal suture. Posterior principal head setae very long, extending beyond base of principal thoracic setae. Principal thoracic setae extending nearly to base of major abdominal setae. Four major abdominal setae; no minute setae on abdomen; no evidence of abdominal segmentation; anal segment not prolonged posteriorly.

*Nymph 2:* (Fig. 20.) Total length 0.45–0.53 mm. Anterior principal head setae minute. Posterior principal head setae conspicuous but relatively much shorter than in nymph 1. Principal thoracic setae about twice as long as posterior principal head setae. Only 2 major abdominal setae. These are located dorsally. Abdomen with anal segment not posteriorly prolonged.

*Nymph 3:* (Fig. 26.) Total length 0.54–0.65 mm. Head, thorax and setal armature similar to nymph 2. Abdomen with 6 major setae. A pair of minute setae both dorsally and ventrally located mesad of posteriormost major abdominals.

*Specimens examined:* 9 first instars, 17 second instars, and 4 third instars from *Oryzomys palustris,* Breton Island, Plaquemines Parish, Louisiana. February 1, 1958.

*Comments:* probably restricted to *Oryzomys* spp.

### *Hoplopleura hirsuta* Ferris

*Nymph 1:* (Fig. 10.) Total length 0.41–0.55 mm. Anterior principal head setae represented by minute setae immediately anterior to the clypeofrontal suture. Posterior principal head setae stout and conspicuous, barely attaining posterior margin of head. Principal thoracic setae approximately 3 times longer than posterior principal head setae, not attaining middle of abdomen. A pair of minute setae on ventral surface of abdomen just posterior to bases of hind coxae. Four major abdominal setae; no trace of abdominal segmentation; anal segment drawn out into a short, tubelike process. No minute setae at posterior end of abdomen.

*Nymph 2:* (Fig. 21.) Total length 0.49–0.67 mm. Head and thorax similar to those of nymph 1 except thoracic principal setae only about 1½ times as long as posterior principal head setae. A small pair of setae on ventral side of abdomen near hind coxal bases. Eight major abdominal setae; no minute setae posteriorly; anal segment not notably prolonged.

*Nymph 3:* (Fig. 27.) Total length 0.74–1.05 mm. Head and thorax similar to those of nymph 2. Abdomen with 3 pairs of minute ventral setae on anterior half. Twelve major abdominal setae; anal segment somewhat prolonged; no minute setae posteriorly on abdomen.

*Specimens examined:* 6 first instars, 9 second instars and 14 third instars from *Sigmodon hispidus,* Breton Island, Plaquemines Parish, Louisiana.

*Comments:* Probably restricted to *Sigmodon* spp.

### *Hoplopleura trispinosa* Kellogg and Ferris

*Nymph 1:* (Fig. 12.) Total length 0.27–0.32 mm. Anterior principal head setae quite small, posterior principal head setae stout, not attaining posterior head margin, no setae near midline dorsally. Principal thoracic setae about 1½ times as long as posterior principal head setae. Abdomen with 4 major setae; a pair of small setae both dorsally and ventrally mesad of base of major abdominals. Anal segment somewhat prolonged posteriorly and bearing 4 small setae. No evidence of abdominal segmentation.

*Nymph 2:* (Fig. 22.) Total length 0.31–0.39 mm. Head and thorax very like those of nymph 1, except there are here 2 pairs of small setae near the thoracic margin and there are 2 pairs of minute setae near the midline of the head dorsally (labeled "X"). Four major abdominal setae plus a small dorsal and ventral pair mesad of base of major abdominals; 4 small setae on apex of anal lobes.

*Nymph 3:* (Fig. 28.) Total length 0.36–0.44 mm. Head and thorax very similar to those of nymph 2. Abdomen with 8 major setae plus a pair of small setae mesad of posteriormost major abdominals both dorsally and ventrally. Six small setae on anal lobe.

*Specimens examined:* 12 first instars, 7 second instars, 6 third instars from 2 *Glaucomys volans,* December 29, 1956, and December 17, 1957, Cedar Creek Forest, Minnesota.

*Comments:* This species is apparently restricted to the genus *Glaucomys.*

### *Hoplopleura arboricola* Kellogg and Ferris

*Nymph 1:* (Fig. 14.) Total length, 0.30–0.35 mm. Anterior principal head setae small; posterior principal stout, extending beyond posterior margin of head. Thoracic principals about 1.5 times as long as posterior principal head setae. Abdomen with 5 pairs of minute spiracles. Posterior half of dorsal side of abdomen with 2 pairs of rather long setae near midline; ventral side with a long and a short pair of setae. Four major abdominal setae. A pair of minute setae near apex of abdomen dorsally, a small seta immediately mesad of base of each major abdominal seta.

*Nymph 2:* (Fig. 23.) Total length, 0.37–0.49 mm. Head and thorax very similar to those of nymph 1. Abdomen with 10 pairs of setae near midline dorsally, with 3 or 4 pairs of setae near midline ventrally. The posteriormost 2 of the latter are the larger. Abdomen with 12 major abdominal setae; a small seta mesad of the base of the posteriormost 4 major abdominals. Minute spiracles present on 5 segments.

*Nymph 3:* (Fig. 31.) Total length 0.41–0.75 mm. Head and thorax very like those of nymph 2. Abdomen with 12 major abdominals; with 10 pairs of setae near midline dorsally and 6 pairs ventrally. Paratergites evident on 4 segments as small sclerotized points. The 1st or the 1st through the 4th paratergites with setae. Abdominal spiracles obvious on 5 segments.

*Specimens examined:* 20 first instars, 21 second instars, 15 third instars from *Eutamias minimus,* Basswood Lake, Minnesota, July 1957; 74 first instars, 115 second instars, and 75 third instars from 3 *Eutamias dorsalis,* Portal, Arizona, July 28–August 8, 1957; 16 first instars, 4 second instars, and 11 third instars from *Eutamias amoenus,* Mt. St. Helens, Washington, August 21, 1956.

*Comments: H. arboricola* is restricted to *Eutamias* spp. in North America. It may actually be a complex since there is some variation in the specimens we have examined from Minnesota,

Washington and Arizona. For example: the 3rd instar specimens from *E. minimus* in Minnesota seem invariably to have 1 seta on each paratergite. Third instars from *E. dorsalis* have setae only on the 1st or 1st and 2nd paratergites. Those from *E. amoenus* have setae on the 1st paratergite in the case of those destined to be males, and on all 4 in those destined to be females. This species is, furthermore, extremely difficult to separate from *Hoplopleura erratica* which is described below. Aside from size, there are practically no differences between these species in the 1st and 2nd instars. The 3rd instars are quite distinctive and the adults can be separated by the following characters in the area where their hosts are in contact.

1. The females of *Hoplopleura arboricola* from *Eutamias minimus* have the posterior margins of paratergite 6 drawn into points posteriorly. Females of *H. erratica* from *Tamias striatus* lack these posterior processes on paratergite 6. This particular character is useful in distinguishing *H. arboricola* from *H. erratica* in the area where the two hosts are in contact (Minnesota). The character is variable on *H. arboricola* from *Eutamias amoenus* and *E. dorsalis*. 2. The females of *H. arboricola* have 4 or more slender sternal sclerites located posteriorly to the 1st sternite of segment 3. The females of *H. erratica* have only 2 or 3 sternites posterior to the 1st sternite of segment 3. 3. Sternal plate width in females of *H. arboricola* in Minnesota range from 0.078 to 0.082 mm with a mean of 0.079 mm. In *H. erratica* this width ranges from 0.085 to 0.094 mm with a mean of 0.092 mm. 4. Sternal plate width in males of *H. arboricola* from Minnesota range from 0.069 to 0.080 mm with a mean of 0.075 mm. In *H. erratica* this width ranges from 0.080 to 0.089 mm with a mean of 0.085 mm. These sternal-plate-width measurements hold very well for specimens of *H. arboricola* from *Eutamias minimus* in Minnesota, and for *H. erratica* from *Tamias striatus* in both Minnesota and Maine. However, specimens of *H. arboricola* from *Eutamias amoenus* in Washington have wider sternal plates which approach the dimensions of the plates in *H. erratica*.

More extensive investigation with long series of specimens from the various host species are required before an adequate clarification of this complex is possible.

### *Hoplopleura erratica* (Osborne)

*Nymph 1:* Total length 0.39–0.51 mm. Identical with nymph 1 of *H. arboricola* except for somewhat larger size.

*Nymph 2:* Total length 0.50–0.65 mm. Essentially identical with nymph 2 of *H. arboricola* except for larger size and smaller number of ventral setae on the abdomen. Only 1 or 2 pairs here rather than the 2 or more pairs present in *H. arboricola*.

*Nymph 3:* (Fig. 30.) Total length 0.63–0.86 mm. Head and thorax identical with nymph 3 of *H. arboricola*. Minute paratergites present on 5 abdominal segments; paratergites without points; no setae present on paratergites. Five pairs of abdominal spiracles. Otherwise identical with *H. arboricola*. Readily separated from *H. arboricola* by the lack of setae on paratergites.

*Specimens examined:* 9 first instars, 8 second instars and 3 third instars from *Tamias striatus*, Waldo Co., Maine, June 28, 1957; 36 first instars, 24 second instars, and 7 third instars from *Tamias striatus*, Basswood Lake, Minnesota, July, 1956.

*Comments:* This species is restricted to *Tamias striatus* in the eastern United States. Records of this from *Eutamia amoenus* in the west must be erroneous identification of *H. arboricola*. (See under *H. arboricola*.)

### *Hoplopleura sciuricola* Ferris

*Nymph 1:* (Fig. 13.) Total length, 0.45–0.53 mm. Anterior principal head setae represented by the inner of a small pair located anterior to the clypeofrontal suture. Posterior principal large, extending beyond posterior margin of head. This seta with a small seta mesad and one laterad of base. Thoracic dorsal principal longer than posterior principal head setae, extending to near middle of abdomen. An anterior-posterior row of pairs of minute seta near midline of ventral side of abdomen. Four major abdominal setae, the ventral pair each with a minute seta mesad of base and each of the dorsal pair with a slightly longer seta mesad of base. A minute pair of setae ventrally near posterior margin. Abdomen with 5 pairs of small spiracles. Cuticle somewhat pigmented and scaly in appearance.

*Nymph 2:* (Fig. 24.) Total length 0.54–0.73 mm. Head and thorax with chaetotaxy like nymph 1 except setae stouter. Two pairs of small marginal setae on thorax, rather than 1 pair, and principal thoracic setae relatively shorter. Ventral side of abdomen with 7 pairs of minute setae extending longitudinally. Dorsal side of abdomen with 3 pairs of setae on anterior third. Twelve major abdominal setae. Each of the posteriormost ventral pairs of major abdominals with a small seta mesad of base; a small pair of setae near posterior end dorsally; a minute pair at apex of abdomen. Abdomen with 5 pairs of small spiracles. Cuticle definitely brownish with a scaled appearance.

*Nymph 3:* (Fig. 29.) Total length 0.66–0.91 mm. Head and thorax like nymph 2. Anterior abdominal seta row with 6 setae rather than 2; posterior ventral major abdominal setae each with 2 minute setae mesad of base. Several small setae at abdominal apex dorsally and ventrally as in the figure. Five pairs of spiracles. Cuticle pigmented and appearing scaled.

*Specimens examined:* 4 first instars, 12 second instars and 12 third instars from *Sciurus carolinensis,* Princeton, Minnesota; 2 second instars and 1 third instar from *Sciurus niger,* Carlos Avery Game Refuge, Minnesota, July 29, 1958.

*Comments:* This species is probably to be found on all North American *Sciurus.* The record from *S. niger* is apparently new.

### *Hoplopleura oenomydis* Ferris

*Nymph 1:* (Fig. 5.) Total length, 0.46–0.57 mm. Anterior principal head setae represented by the inner of a small pair located anterior to the clypeofrontal suture. Posterior principal obvious, but not especially long, barely attaining posterior head margin. A small seta mesad of base of posterior principal. Principal thoracic setae about 1½ times as long as posterior principal head setae. A minute pair of setae on venter of abdomen near posterior coxae; another small pair on venter near posterior end of abdomen. Only 2 major abdominal setae. Dorsal side of abdomen with a row of 6 pairs of sclerites.

*Nymph 2:* (Fig. 15.) Total length, 0.56–0.71 mm. Head and thorax like those of nymph 1, except 2 pairs of marginal thoracic setae rather than 1 pair. Abdomen without major setae. A longitudinal row of pairs of minute setae on ventral side of abdomen; 3 pairs of minute setae at apex ventrally, 1 pair at apex dorsally.

*Nymph 3:* (Fig. 25.) Total length, 0.66–0.96 mm. Practically identical with nymph 2, except for a single pair of small setae dorsally and ventrally near posterior end of abdomen (labeled "X").

*Specimens examined:* 3 first instars, 4 second instars, and 5 third instars from "Rats" Atlanta, Ga. (no other data); 10 first instars, 10 second instars, 9 third instars, from *Rattus,* Atlanta, Ga., December, 1946.


## SUMMARY

The nymphal stages of the known North American species of the genus *Hoplopleura* are described and illustrated. Two new species are described, *H. ferrisi* and *H. onychomydis.* The former can only be recognized in the immature stages. Characters that distinguish *H. arboricola* K. and F. and *H. erratica* (Osb.) in both immature and adult stages are discussed. Keys to all immature stages are presented.


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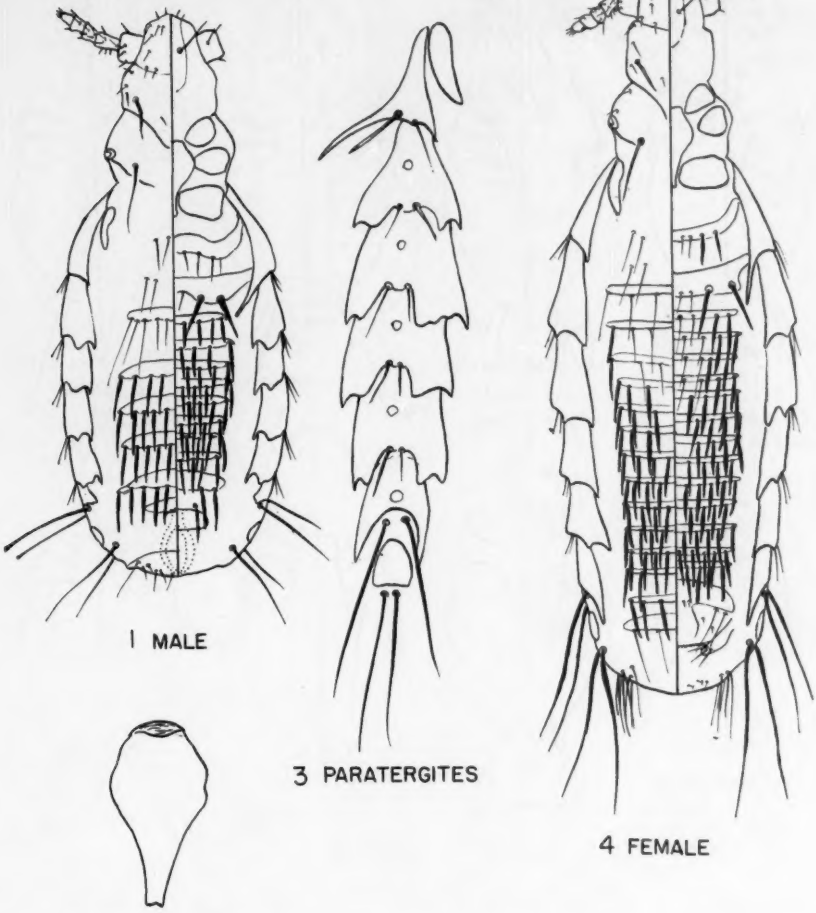
## Explanation of Plates

Figures 1–4. *Hoplopleura onychomydis,* n. sp.
Figures 5–14. First instars of *Hoplopleura* spp.
Figures 15–24. Second and third instars of *Hoplopleura* spp.
Figures 25–31. Third instars of *Hoplopleura* spp.

PLATE I



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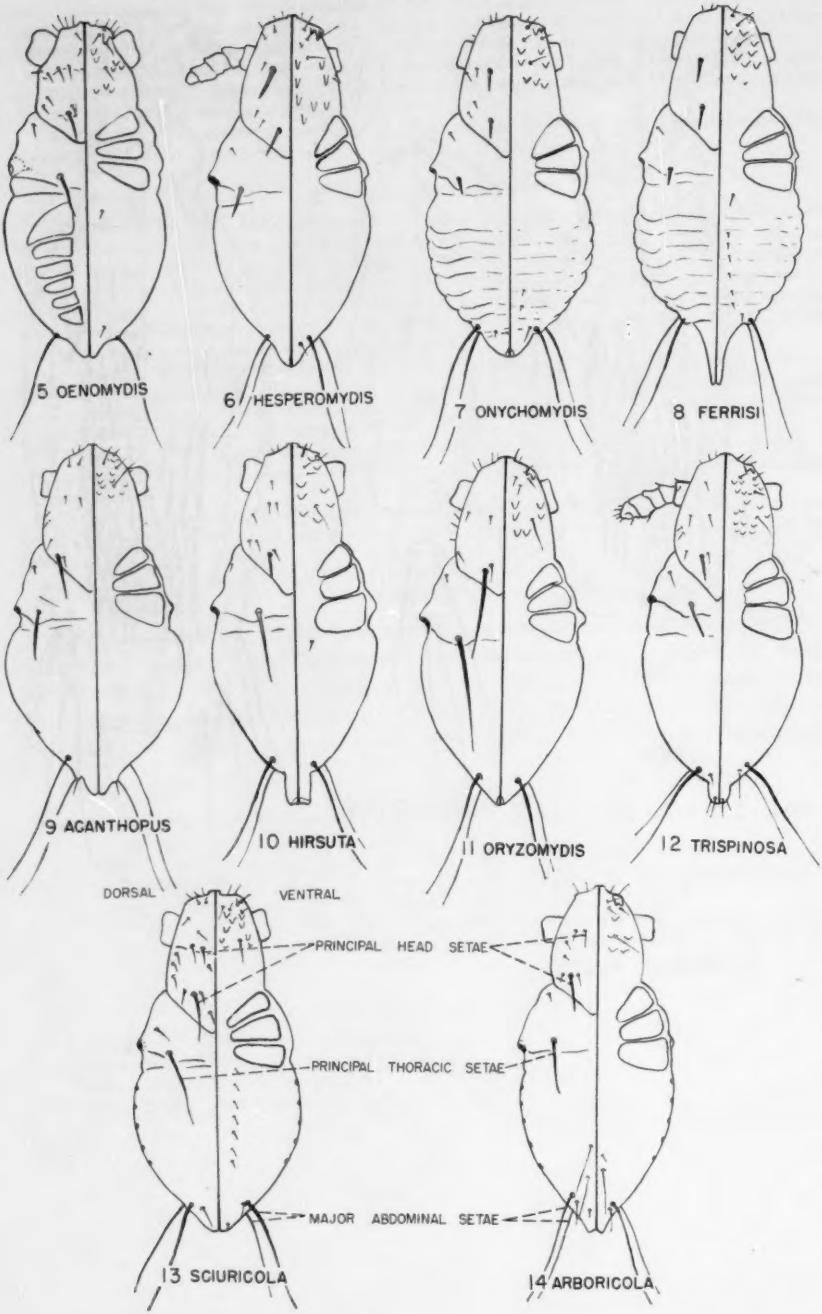
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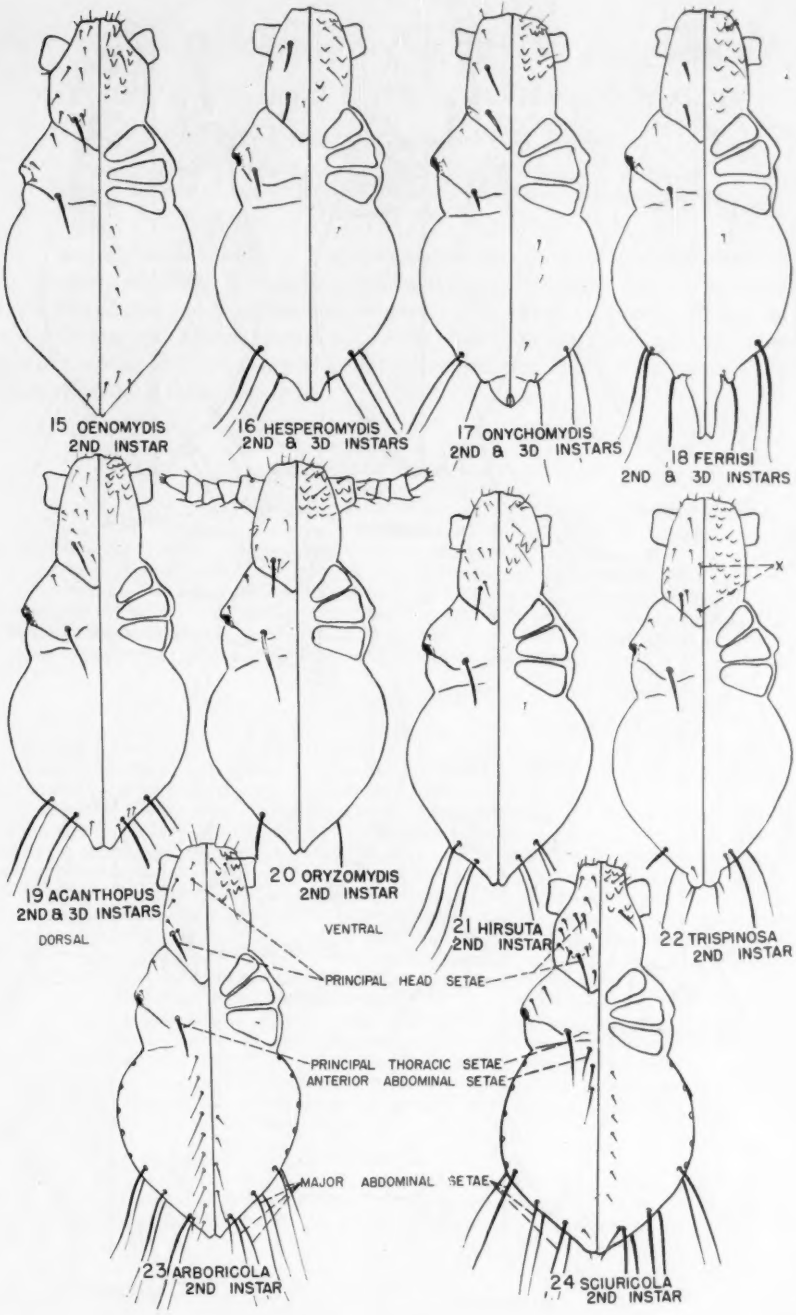
6 HESPEROMYDIS
7 ONYCHOMYDIS
8 FERRISI
9 ACANTHOPUS
10 HIRSUTA
11 ORYZOMYDIS
12 TRISPINOSA
DORSAL
VENTRAL
PRINCIPAL HEAD SETAE
PRINCIPAL THORACIC SETAE
MAJOR ABDOMINAL SETAE
13 SCIURICOLA
14 ARBORICOLA

PLATE III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17 ONYCHOMYDIS
2ND & 3D INSTARS
18 FERRISI
2ND & 3D INSTARS
X
19 ACANTHOPUS
2ND & 3D INSTARS
DORSAL
20 ORYZOMYDIS
2ND INSTAR
VENTRAL
21 HIRSUTA
2ND INSTAR
22 TRISPINOSA
2ND INSTAR
PRINCIPAL HEAD SETAE
PRINCIPAL THORACIC SETAE
ANTERIOR ABDOMINAL SETAE
MAJOR ABDOMINAL SETAE
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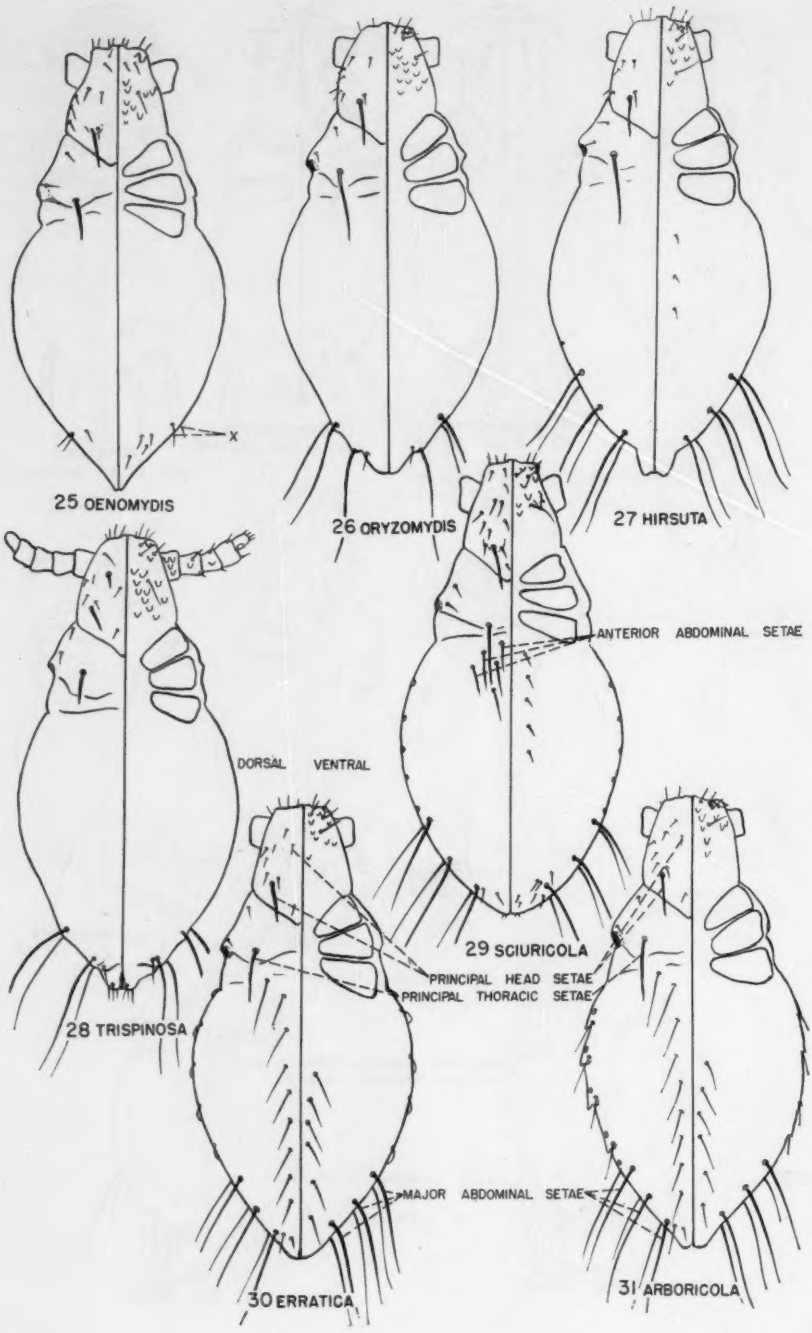
2ND INSTAR
24 SCIURICOLA
2ND INSTAR

PLATE IV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X
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ANTERIOR ABDOMINAL SETAE
DORSAL
VENTRAL
28 TRISPINOSA
29 SCIURICOLA
PRINCIPAL HEAD SETAE
PRINCIPAL THORACIC SETAE
MAJOR ABDOMINAL SETAE
30 ERRATICA
31 ARBORICOLA

# THE *HAEMAPHYSALIS* TICKS (ACARINA: IXODIDAE) OF BIRDS.

## I. *H. ORNITHOPHILA* N. SP. FROM BURMA AND THAILAND*

HARRY HOOGSTRAAL AND GLEN M. KOHLS

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Hamilton, Montana

Owing to ever increasing evidence concerning the role of birds and their parasites in the spread of viruses pathogenic to man and domestic animals, we have been making a special study of ticks that feed on birds. The present report is the first in a series dealing with *Haemaphysalis* ticks from birds. We are grateful to the authorities of the Harvard University Museum of Comparative Zoology for allowing us to study material in their collection.

*Haemaphysalis ornithophila* n. sp.
(Figures 1 to 12)

*Holotype.* Male, from *Pitta oatesi oatesi* (Hume, 1873) (Burmese Rufous-naped Pitta), Doi Angka, Siam (Thailand), between 28 February and 18 April, 1937, H. Coolidge Jr., *legit.* Deposited in the Museum of Comparative Zoology, Harvard University.

*Allotype.* Female, data as for holotype, also in Museum of Comparative Zoology.

*Paratypes.* Two males with data as above, 1 in Museum of Comparative Zoology and 1 in Hoogstraal collection. One male (RML 22847) from *Gallus gallus gallus* (Linnaeus, 1758) (Red Jungle Fowl) Myitkyina, Burma 13 May 1945, G. E. Davis, United States of America Typhus Commission, *legit.;* deposited in Rocky Mountain Laboratory.

### DESCRIPTION

MALE. Holotype, overall length, 2.1 mm, width 1.3 mm. Thailand paratypes 2.1 mm by 1.4 mm and 2.3 mm by 1.3 mm; Burma paratypes 2.0 mm by 1.4 mm. Body widest immediately anterior to spiracular plates, lateral margins rather acutely converging from midlength of scutum to scapulae; posterior margin broadly rounded. Color yellowish brown.

*Capitulum.* Basis capituli rectangular, lateral margins parallel or only very slightly diverging anteriorly, posterior margin straight; cornua gradually tapering to an acute apex, approximately ¼ as long as basis capituli. Palpi broadly salient basally, basal margins straight and lacking either dorsal or ventral spurs, lateral margins meeting basal salience at almost right angles; segment 3 approximately 4/5 as long as segment 2 and ventrally bearing a long thin, sharply pointed spur reaching almost to midlength of segment 2. Inner margin of palpi fairly straight; dorsobasal margin of segment 3 straight, lacking spur. Hypostome narrowly elongate, lateral margins slightly convex, apex bluntly rounded; a slight corona present; dentition 4/4 in files of 8 or 9 denticles, denticles narrowly elongate and sharply pointed apically.

*Scutum.* Lateral grooves deep and broad, extending from level of middle of coxa III and including 1 festoon on each side. Cervical grooves deep, wide, and straight anteriorly, extending as shallow, narrow, broadly diverging grooves reaching approximately the same level as the anterior termination of lateral grooves. Festoons clearly marked, numbering 11, elongate. Punctations quite numerous; fairly large, uniform, and deep, mostly noncontiguous; uniformly distributed over scapulae and scutal surface except for a band across anterior third; a few small punctations on festoons and on lateral margins.

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*Legs.* Coxa I with a strong, sharply pointed spur arising from middle of posterior margin and extending to anterior margin of coxa II; spur of II as an angled ridge terminating at middle of posterior margin in a sharp point; spur of III similar to that of II but ridge only slightly diverging from inner margin of coxa and very slightly overlapping posterior margin of coxa; spur of IV similar to that of III. Trochanter I dorsally with a moderate shield, ventrally with a strong, pointed spur arising distally from the posterior margin; II to IV with faint ridges suggestive of a spur ventrally. Tarsi elongate, gradually tapering, apicodorsal margin of IV slightly more concave than that of others, not humped, lacking apicoventral hook. Claws long, narrow, sharply curved over apices of pads.

*Ventral surface.* Integument with short, regularly spaced hairs as illustrated. Genital aperture large, situated level with midlength of coxa II. Genital and anal grooves distinct, as illustrated. Spiracular plates subtriangular, as illustrated.

FEMALE. Allotype, greatly engorged. In nonsecondary characters, this sex resembles the male. Length from posterior margin of scutum to apex of palpi approximately 1.6 mm; width of scutum approximately 1.1 mm; total length engorged approximately 5.2 mm.

*Capitulum.* Basis capituli with cornua slightly shorter than those of male; porose areas large, subcircular. Palpi as in male but more elongate, segment 2 being slightly longer in relation to segment 3, basolateral salience slightly more elongate, and juncture of lateral and basal margins less acute. Hypostome with outline appearing as in male but slightly more elongate (damaged apically in this specimen); dentition appearing to be 5/5.

*Scutum.* Subcircular with gradually rounded margins, length (including scapulae) and width approximately equal. Cervical grooves deep and straight, extending beyond midlength of scutum. Punctations as in male, somewhat more numerous on posterior third of scutum than elsewhere.

*Legs.* Coxae slightly more rectangular but otherwise similar to those of male; spurs also similar except that that of II also extends slightly beyond basal margin of coxa, as on III and IV; spur I equally large and arising from same position as in male. Trochanters, tarsi, pads, and claws as in male.

*Ventral surface* and *spiracular plates.* As illustrated.

IMMATURE STAGES. Unknown.

*Related species.* This is a member of the *doenitzi* group of bird-parasitizing haemaphysalids of Asia, Madagascar, and Africa including *H. doenitzi* Warburton and Nuttall, 1909; *H. centropi* Kohls, 1949; *H. minuta* Kohls, 1950; *H. pavlovskyi* Pospelova-Shtrom, 1935; *H. madagascariensis* Colas-Belcour and Millot, 1948 (new combination), and *H. hoodi* Warburton and Nuttall, 1909. *H. ornithophila* n.sp. differs from all of these in the strongly developed ventral spurs of palpal segment 3, of coxa I, and of trochanter I, as well as in other characters.

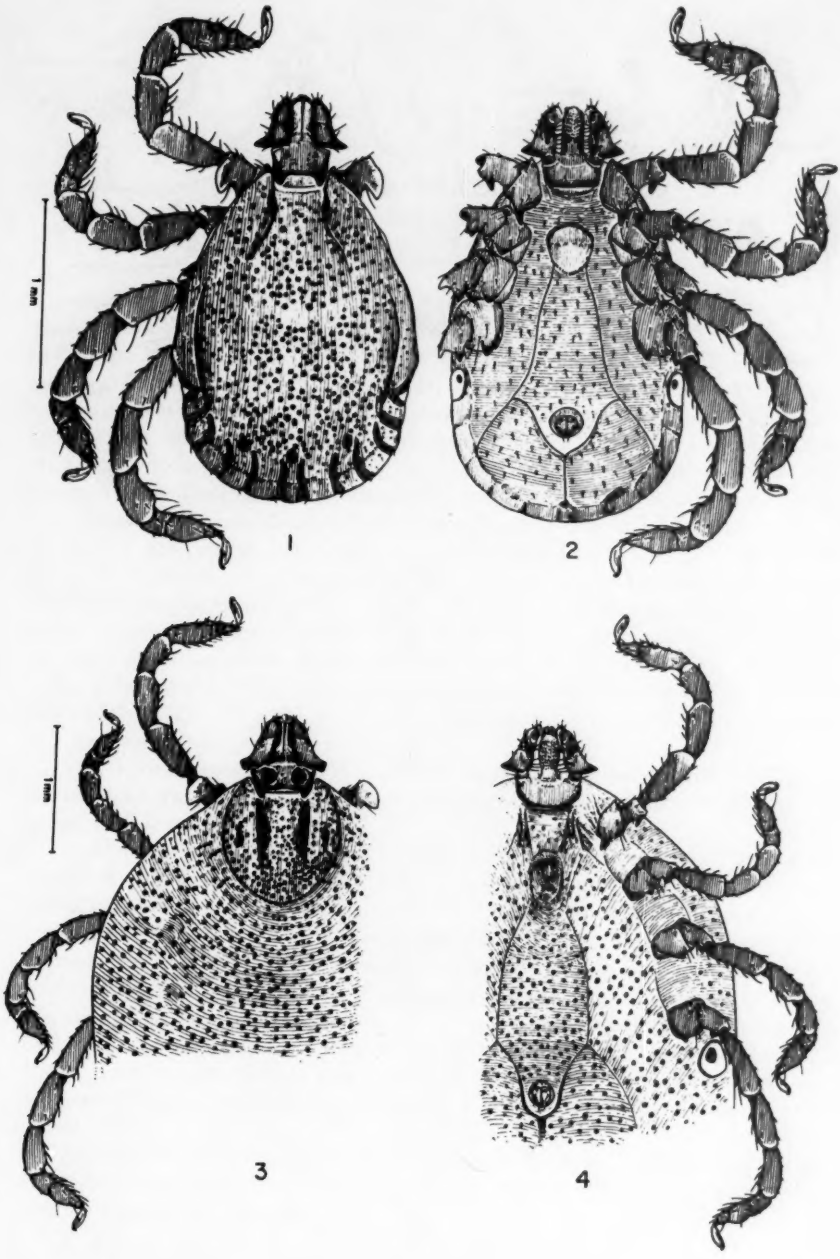
## SUMMARY

Both sexes of *Haemaphysalis ornithophila* n. sp. are described from the birds, *Pitta o. oatesi* (Hume, 1873) from Thailand, and *Gallus g. gallus* (Linnaeus, 1758) from Burma. The new species, a member of the *doenitzi* group of bird ticks of Asia, Madagascar, and Africa, has exceptionally strong ventral spurs on palpal segment 3, coxa I, and on trochanter I.

## EXPLANATION OF FIGURES

*Haemaphysalis ornithophila* n. sp. (holotype and allotype)

FIGURES 1 and 2. Male, dorsal and ventral views.
FIGURES 3 and 4. Female, dorsal and ventral views.
FIGURE 5. Male, basis capituli and palpus, dorsal and ventral views.
FIGURE 6. Male, hypostome, ventral view.
FIGURE 7. Male, spiracular plate.
FIGURE 8. Male, tarsi I to IV, lateral view.
FIGURE 9. Female, basis capituli and palpus, dorsal and ventral views.
FIGURE 10. Female, hypostome, ventral view.
FIGURE 11. Female, spiracular plate.
FIGURE 12. Female, tarsi I to IV, lateral view.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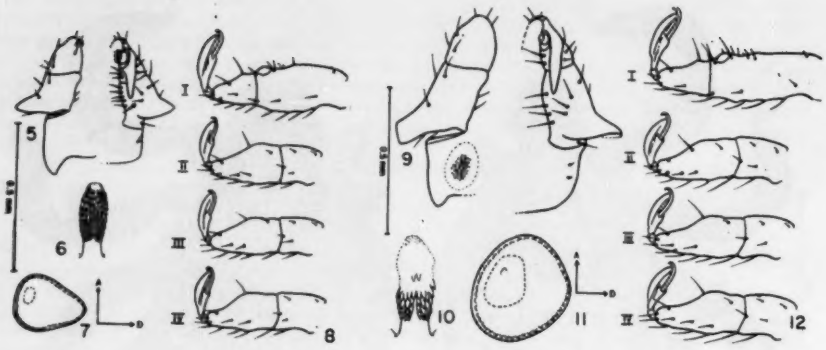
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# *PSEUDOSCHÖNGASTIA* AND FOUR NEW NEOTROPICAL SPECIES OF THE GENUS (ACARINA: TROMBICULIDAE)

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Since Lipovsky (1951) described *Pseudoschöngastia,* this elusive genus has already experienced a colorful taxonomic career. On the basis of 7–6–6 leg segmentation in the larvae, Lipovsky placed it in the Gahrliepiinae (= Walchiinae). Brennan (1952), Wharton and Fuller (1952), and Gould (1956) endorsed this action.

Noting that members of subfamily Gahrliepiinae lack an AM (present in *Pseudoschöngastia*), Womersley (1954) regarded the group as trombiculine and, questioning the generic importance of extrascutal PL's and 7–6–6 leg segmentation in odd forms of Trombiculinae, synonymized the genus under *Euschöngastia.* He added that larvae of *Pseudoschöngastia* and *Ascoschöngastia* cannot be separated.

Audy (1954) recognized the extrascutal PL's and femoral fusions in legs II and III as recurrent polyphyletic characters and placed *Pseudoschöngastia* in the Trombiculinae, but treated the group as a distinct genus with the qualification that its status requires more confirmation. Loomis (1956) concurred and suggested that the fused condition of the femora may not even represent a character of generic value. He also noted that in *Pseudoschöngastia* the number of branched setae on femora II and III are 6 and 5 as in other trombiculine genera, but that the Gahrliepiinae have fewer setae on these segments.

Vercammen-Grandjean (1956), without explanation, merely stated that Lipovsky found an *Ascoschöngastia* with 7–6–6 leg segmentation and described it as a new genus, *Pseudoschöngastia.*

Audy (1957), relating *Pseudoschöngastia* to *Ascoschöngastia* and *Laurentella,* suggested that these form a complex and might even be congeneric. He further stated that nymphs of *Laurentella, Ascoschöngastia,* and *Pseudoschöngastia* were indistinguishable. Then, Audy and Womersley (1957), recounting shared characters of these groups and others, mentioned reliable generic characters in *Pseudoschöngastia* larvae such as short, thickened leg segments, setation of palpal tarsus, and other characters of no more than specific value, such as condition of galeal and palpal setae, size of scutum, position of AM with respect to AL, number of genualae I, etc. Finally, they declared that *Pseudoschöngastia* nymphs do differ from those of *Ascoschöngastia* and *Laurentella* in having a complete sternum and subequal paracristal setae.

With the availability of more material and in the light of discussions in literature cited above, we regard *Pseudoschöngastia* as a valid trombiculine genus, our concept of which is detailed below.

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We place little emphasis on either the fused femora of legs II and III, or on the extrascutal PL's. However, among those species which we consider typical members of *Pseudoschöngastia,* at least 1 of these 2 conditions obtains. Characters which appear sound are setation of palpal tarsus, short thick legs, and ventral humeral setae between coxae II and III.

Whether femora II and III are joined or divided is frequently a matter of interpretation. This is admirably illustrated in the closely related Hoffmann species *P. anomala, diazi,* and *pedregalensis.* However, no matter how rigidly basifemur and telofemur are joined, the line of articulation is usually visible. Thus femoral fusion in *Pseudoschöngastia* (Trombiculinae) does not show the degree of perfection achieved by the Leeuwenhoekiinae, but does approach that of the Gahrliepiinae.

As Audy and Womersley (1957) have noted, the center of development is neotropical. Furthermore, according to our interpretation, the genus is not represented in Old World fauna. The larvae parasitize small mammals including rodents, lagomorphs, and insectivores. Relationships of *Pseudoschöngastia* to other genera are not clear. To do more than suggest an affinity with *Euschöngastia,* in the broadest sense, would be sheer speculation. There follows a redescription of the genus and a summary of its species.

*Pseudoschöngastia* Lipovsky, 1951

Larvae of the subfamily Trombiculinae. Palpal tarsus with 5 branched setae and a tarsala. Palpal claw trifurcate. Legs short and thickened. Ventral humeral setae between coxae II and III. PL's on or off scutum, but usually extrascutal. Sensillae broadly expanded. Eyes present. Femora of legs II and III fused, semi-fused, or divided. Body multisetose, usually with more than 1 pair of humerals.

*P. hungerfordi* Lipovsky, 1951, type species. Holotype off *Sylvilagus,* other material from numerous small mammals; Kansas, Missouri, and Texas, U.S.A.

*P. farneri* Lipovsky, 1951. Holotype off *Cryptotis,* other material from rodents, insectivores and lagomorphs; Kansas, Colorado, U.S.A.

*P. diazi* (Hoffmann, 1948). Holotype off "'tepeizcuinte' (pequeño roedor cuya especie no fué determinada)", other specimens from *Peromyscus*; Chiapas, Mexico. In the English summary "roedor" is translated as "badger" but actually the word implies "rodent"; moreover it is unlikely that the badger, known in northern Mexico, occurs as far south as Chiapas. In Central America "tepescuinte" is the paca (*Cuniculus*), a *large* edible rodent, a species of which does occur in southern Mexico. Ventral humeral setae between coxae II and III were neither described nor figured by Hoffman. However, in a paratype recently seen, 2 are present on each side. This species, transferred by Lipovsky (1951) to *Pseudoschöngastia,* was described as an *Ascoschöngastia* by Hoffmann. She noted femoral fusion in legs II and III, thus becoming the first to observe this condition in the Trombiculinae.

*P. anomala* (Hoffmann, 1951), new combination. Holotype off *Peromyscus,* other material from *Sorex* and *Peromyscus;* Mexico, D. F., Mexico.

*P. pedregalensis* (Hoffmann, 1951), new combination. Holotype off *Baiomys,* other material from *Peromyscus* and *Sorex;* Mexico, D. F., Mexico.

Hoffmann also placed the last 2 species in *Ascoschöngastia* on the basis of extrascutal PL's and divided femora II and III shortly before the creation of *Pseudoschöngastia* of which they are typical representatives. This incidentally was hinted by Loomis (1956) when he stated, "additional species, now considered *Ascoschöngastia,* from Mexico and the Old World almost certainly belong to this genus." We (1959) questioned their distinctness, but from later studies are convinced that these forms, though close, are separable. We find that *anomala* characteristically has 3 genualae I, not 2 as described, and *pedregalensis* has 2 genualae I, as described. Other differences, possibly not all of them specific, are as given by Hoffmann.

*P. occidentalis* Brennan, 1952. Holotype off *Peromyscus,* few other specimens from *Peromyscus;* California, U.S.A.

*P. guatemalensis* Brennan, 1952. Holotype off *Sylvilagus,* other specimens from *Sylvilagus;* Chimaltenango, Guatemala.

*P. novita* Audy and Womersley, 1957. Holotype off *Rattus*, other material from *Rattus* and *Tupaia*; Selangor, Malaya. We have not seen specimens, but from the description and figures the species is not typical of *Pseudoschöngastia* and is very probably misplaced generically. The palpal tarsus has only 4 branched setae. Eyes are absent. No mention is made of ventral humeral setae; but, since all other setae are enumerated, we assume these to be absent. The small number and form of body setae are not characteristic of *Pseudoschöngastia*. Sensillae are missing from all specimens recorded.

*P. audyi*, n.sp. Holotype off *Peromyscus*, other specimens from same host; Sacatepequez, Guatemala.

*P. aberrans* n.sp. Holotype off *Peromyscus;* Chiapas, Mexico.

*P. hypopsia* n.sp. Holotype off *Baiomys;* Chiapas, Mexico.

*P. scitula* n.sp. Holotype off *Liomys;* Chiapas, Mexico.

## DESCRIPTIONS OF NEW SPECIES

### *Pseudoschöngastia audyi* n.sp.

(FIGURE 1)

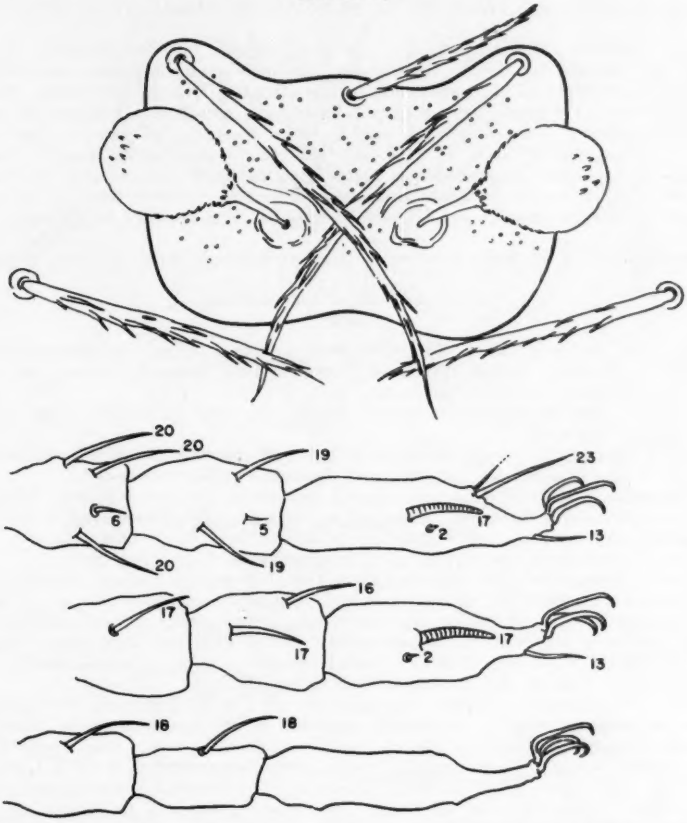


FIGURE 1. *P. audyi* n.sp. Scutum; specialized leg setae showing relative lengths in microns.

*Type data.* Holotype and 9 paratypes, RML No. 34060, off white-footed mouse, *Peromyscus g. guatemalensis*, Finca San Rafael, 6900 feet, Sacatepequez, Guatemala, 23 June 1948, R. D. Mitchell and Luis de la Torre, collectors.

Holotype and some paratypes in the Chicago Natural History Museum, other paratypes in the Rocky Mountain Laboratory, the United States National Museum and the British Museum (Natural History).

*Diagnosis.* Resembles *P. hungerfordi* from which it differs by the very long microgenuala and microtibiala on leg I, generally longer specialized leg setae, tarsala I as long as tarsala II, longer and fewer dorsal setae, setules of sensillae nearly imperceptible, and AL much longer in proportion to AM and PL.

*Body.* Ellipsoidal. Length and width of holotype, partly engorged, 490 × 281 microns. Eyes 2/2, no plate. Anus at about 5th row of ventral setae.

*Gnathosoma:* Cheliceral bases and capitular sternum punctate. Blades with small tricuspid cap and dorsal and ventral tooth. Palpal setal formula B/B/NNN(b), the branched ones with few branches. Claw trifurcate. Palpal tarsus with a moderately long tarsala and 5 branched setae. Galeal seta nude.

*Scutum.* Wider than long with sinuous anterior margin and rounding, emarginate posterior margin; punctate. Sensillary bases fairly close together. Sensillae capitate, setules weak, barbules part way down stems. Setae with appressed barbs. AM posterior to AL's. Scutal measurements of holotype: AW-58, PW-91, SB-18, ASB-26, PSB-15, AP-37, AM-27, AL-73, PL-40, S-34.

*Legs.* Punctate. *Specialized setae:* Leg I—3 genualae, long microgenuala; 2 similar tibialae, long microtibiala; tarsala, microtarsala, sub- and parasubterminala, pretarsala. Leg II—genuala; 2 tibialae; tarsala, microtarsala, pretarsala. Leg III—genuala; tibiala. *Branched setae:* Leg I—coxa 1, trochanter 1, basifemur 1, telofemur 5, genu 4, tibia 8, tarsus 18. Leg II—coxa 1, trochanter 1, femur 6, genu 4, tibia 6, tarsus 16. Leg III—coxa 1, trochanter 1, femur 5, genu 3, tibia 6, tarsus 14. All tarsi with paired claws and a slender empodium.

*Body setae.* Dorsal setae similar to scutal setae, 31 to 39 microns long, decreasing in length medially and posteriorly, 2-2 humerals plus 42 to >60. Ventral setae 2-2 sternals and 2-2 humerals between coxae II and III plus 40 to >50, those posterior to the anus like the dorsals.

Named for Dr. J. R. Audy, Institute for Medical Research, Kuala Lumpur, Malaya.

*Pseudoschöngastia aberrans* n.sp.
(FIGURE 2)

*Type data.* Holotype, RML No. 34930, off white-footed mouse, *Peromyscus oaxacensis*, 6 km NW of Teopisca, Chiapas, Mexico, 1 September 1953, Deane P. Furman and Douglas Price. In the Rocky Mountain Laboratory.

*Diagnosis.* Leg segmentation apparently 7-6-6. PL setae on scutum. One genuala I and no genualae II and III.

*Body.* Subquadrate. Length and width of holotype, engorged, 515 by 402 microns. Eyes small, 2/2. Anus between 5th and 6th rows of ventral setae.

*Gnathosoma.* Cheliceral bases and capitular sternum with scattered puncta. Blades with minute tricuspid cap and also a small dorsal and ventral tooth. Palpal setal formula B/N/NNB, the branched ones with only 3 or 4 branches; genual seta very long. Claw trifurcate. Palpal tarsus with a moderately long tarsala and 5 branched setae. Galeal seta nude.

*Scutum.* Deep, little wider than long, with rounding, slightly indented posterior margin. Puncta conspicuous, but rather sparsely scattered. Deep crescentic folds in front of sensillary bases. Sensillae broad-ovate, heavily armed with long, strong setules on both surfaces, smaller barbules continuing part way down long stems. Scutal setae with long barbs, longest on AL's and AM. AM posterior to AL's. Scutal measurements of holotype: AW-32, PW-44, SB-20, ASB-20, PSB-19, AP-26, AM-22, AL-., PL-23, S-33.

*Legs.* All segments punctate. *Specialized setae:* Leg I—1 genuala, microgenuala; 2 tibialae, microtibiala; tarsala, microtarsala proximal to it, parasubterminala, subterminala, pretarsala. Leg II—no genuala; 2 tibialae; tarsala considerably shorter than tarsala I, microtarsala, pretarsala. Leg III—no genuala; tibiala. *Branched setae:* Leg I—coxa 1, trochanter 1, basifemur 1, telofemur 5, genu 4, tibia 8, tarsus 18. Leg II—coxa 1, trochanter 1, femur 6, genu 4, tibia 6, tarsus 16. Leg III—coxa 1, trochanter 1, femur 5 (a ventral seta of this group delicate and filiform with only one or two weak barbs), genu 3, tibia 6, tarsus 14. All tarsi with paired claws and a slender empodium.

*Body setae.* Dorsal setae pectinate, similar to posterolateral scutal setae, 23 to 39 microns long, decreasing in length medially and posteriorly. Dorsal formula of holotype: 4-8-6-8-8-6-4. Ventral setae 2-2 sternals and 2-2 humerals between coxae II and III plus 36, those posterior to the anus similar to the dorsals.

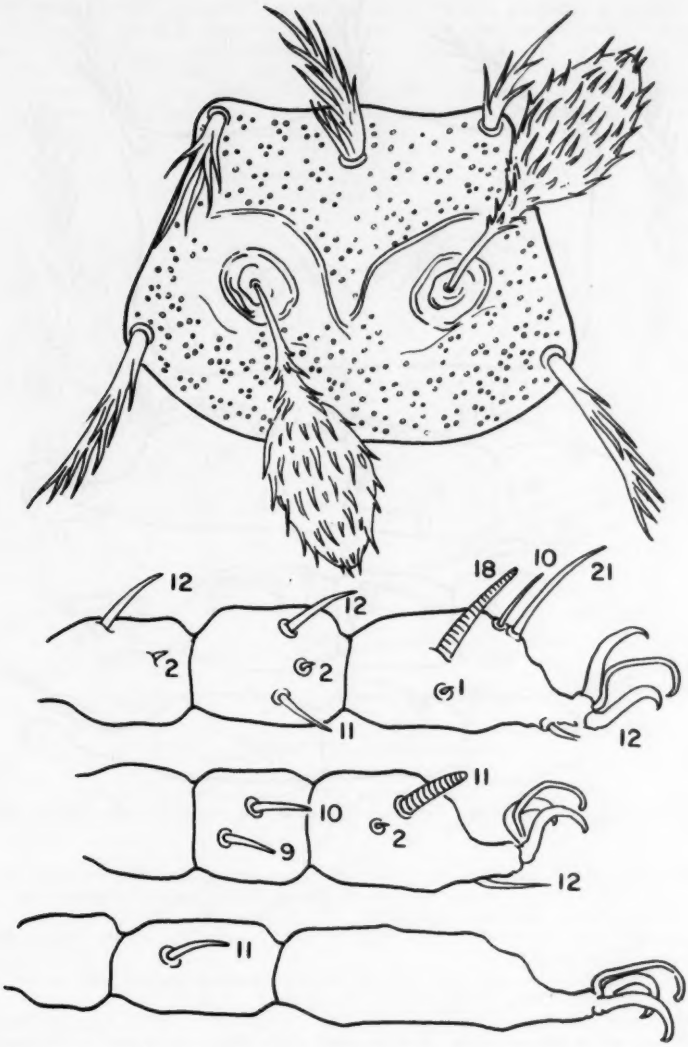


FIGURE 2. *P. aberrans* n.sp. Scutum; specialized leg setae showing relative lengths in microns.

*Pseudoschöngastia hypopsia* n.sp.
(FIGURE 3)

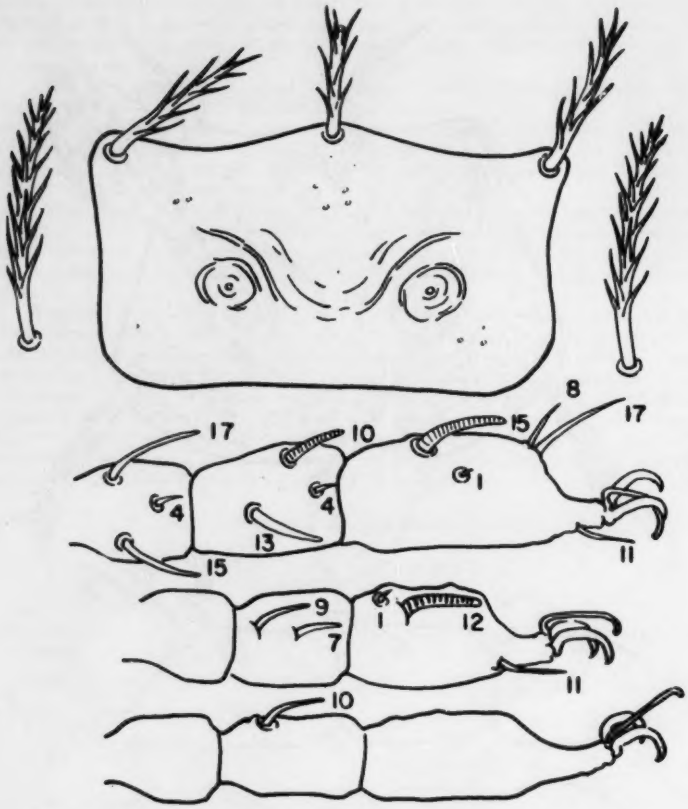


FIGURE 3. *P. hypopsia* n.sp. Scutum; specialized leg setae showing relative lengths in microns.

*Type data.* Holotype, RML No. 34931, off pigmy mouse, *Baiomys musculus nigrescens,* 11 km. NNW of Tuxtla, Chiapas, Mexico, 26 August 1953, Deane P. Furman and Douglas Price. In the Rocky Mountain Laboratory.

*Diagnosis.* Leg segmentation apparently 7-7-7. PL setae off scutum. Two genualae I and no genualae II and III.

*Body.* Ellipsoidal. Length and width of holotype, partly engorged, 355 by 210 microns. Eyes 2/2, in a plate. Anus at 5th row of ventral setae.

*Gnathosoma.* Cheliceral bases and capitular sternum punctate. Cheliceral blades with tricuspid cap and a ventral tooth. Palpal setae: B/N/NNB, the genual very long. Claw trifurcate. Palpal tarsus with 5 branched setae and a tarsala. Galeal seta nude.

*Scutum.* Wider than long, with sinuous anterior margin and relatively straight posterior margin, indistinctly punctate. Sensillary bases well separated and a fold in front of each. Sensillae missing. Setae of similar form with semi-appressed long barbs. AM slightly anterior to level of AL's. Scutal measurements of holotype: AW-52, PW-69, SB-25, ASB-19, PSB-13, AP-26, AM-. ., AL-17, PL-29, S-. . .

*Legs.* All segments punctate. *Specialized setae:* Leg I—2 genualae, microgenuala; 2 tibialae, similar in form, microtibiala; tarsala, microtarsala, sub- and parasubterminala, pretarsala. Leg II—no genuala; 2 tibialae; tarsala, microtarsala, pretarsala. Leg III—no genuala; tibiala. *Branched setae:* Leg I—coxa 1, trochanter 1, basifemur 1, telofemur 5, genu 4, tibia 8, tarsus 18. Leg II—coxa 1, trochanter 1, basifemur 2, telofemur 4, genu 3, tibia 6, tarsus 15. Leg III—coxa 1, trochanter 1, basifemur 2 (the posterior one with fewer branches and more delicate than the other), telofemur 3, genu 3, tibia 6, tarsus 13. All tarsi with paired claws and a slender empodium.

*Body setae.* Dorsal setae similar to scutal setae, 17 to 25 microns long, 2-2 humerals plus about 80. Ventral setae 2-2 sternals and 2-2 humerals between coxae II and III plus about 60, those posterior to the anus similar to the dorsals.

*Pseudoschöngastia scitula* n.sp.
(FIGURE 4)

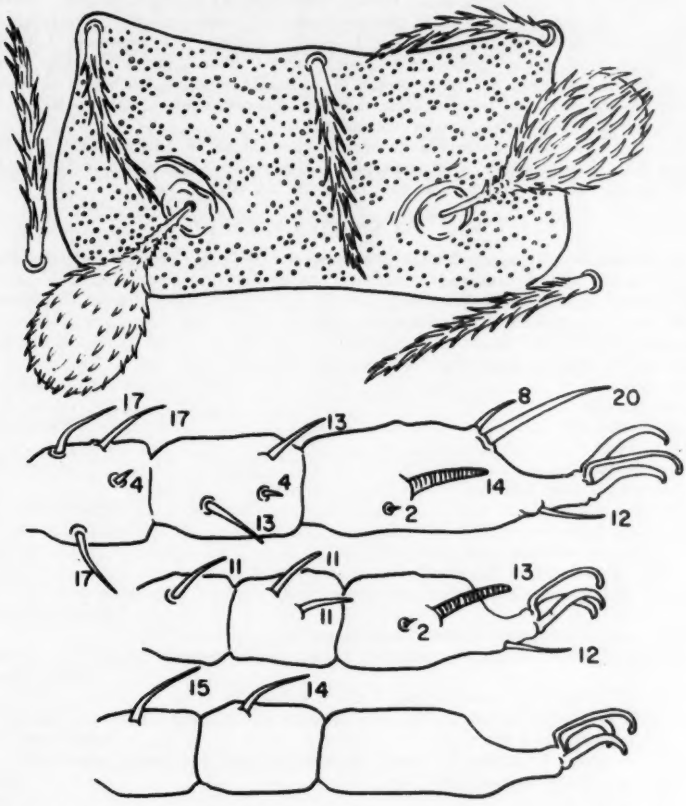


FIGURE 4. *P. scitula* n.sp. Scutum; specialized leg setae showing relative lengths in microns.

*Type data.* Holotype, RML No. 34933, off spiny pocket mouse, *Liomys pictus isthmius,* Pan American Highway at Rio San Gregorio, Chiapas, Mexico, 6 September 1953, Deane P. Furman and Douglas Price. In the Rocky Mountain Laboratory.

*Diagnosis.* Leg segmentation apparently 7-6-6. Three genualae I, and genualae II and III present. PL setae off scutum. AM scutal seta longer than AL. Palpal genual seta long, nude.

*Body.* Oval. Length and width of holotype, virtually unengorged, 231 by 132 microns. Eyes 2/2, in a plate; the anterior one slightly smaller than the posterior. Anus at about 5th row of ventral setae.

*Gnathosoma.* Cheliceral bases and capitular sternum punctate. Blades with small tricuspid cap and a conspicuous dorsal and ventral tooth. Palpal setae: B/N/NNN, the genual unusually long. Claw trifurcate. Palpal tarsus with a slender tarsala and 5 branched setae. Galeal seta nude.

*Scutum.* Wider than long, with sinuous anterior and slightly concave posterior margins; punctate. Sensillary bases widely separated. Sensillae subcapitate with setules evenly distributed on both surfaces, those of the anterior surface the longer; stout barbules extend at least halfway down stems. Scutal setae all of similar form with semi-appressed barbs. AM posterior to level of AL's. Scutal measurements of holotype: AW-57, PW-72, SB-33, ASB-22, PSB-13, AP-31, AM-28, AL-24, PL-34, S-28.

*Legs.* All segments punctate. *Specialized setae:* Leg I—3 genualae, microgenuala; 2 tibialae, similar in form, microtibiala; tarsala, microtarsala, parasubterminala, subterminala, pretarsala. Leg II—genuala; 2 tibialae; tarsala, microtarsala, pretarsala. Leg III—genuala; tibiala. *Branched setae:* Leg I—coxa 1, trochanter 1, basifemur 1, telofemur 5 (a ventral seta of this group appears to be nude), genu 4, tibia 8, tarsus 18. Leg II—coxa 1, trochanter 1, femur 6, genu 3, tibia 6, tarsus 16. Leg III—coxa 1, trochanter 1, femur 5 (a posteroventral one nude and more delicate than the others), genu 3, tibia 6, tarsus 14. All tarsi with paired claws and a slender empodium.

*Body setae.* Dorsal setae similar to scutal setae, 25 to 28 microns long, 2-2 humerals plus about 80. Ventral setae 2-2 sternals and 2-2 humerals between coxae II and III plus about 60, those posterior to the anus similar to the dorsals.

## KEY TO SPECIES LISTED

1. Palpal tarsus with 4 branched setae, eyes absent ..... *novita* Audy & Womersley
   Palpal tarsus with 5 branched setae, eyes present ..... 2
2. One genuala I, PL setae on scutum ..... *aberrans* n.sp.
   More than one genuala I, PL setae off scutum ..... 3
3. Genualae II and III absent ..... *hypopsia* n.sp.
   Genualae II and III present ..... 4
4. Two genualae I ..... 5
   Three genualae I ..... 8
5. Sternal setae 2-4 ..... *occidentalis* Brennan
   Sternal setae 2-2 ..... 6
6. Palpal genual seta nude ..... *farneri* Lipovsky
   Palpal genual seta branched ..... 7
7. Galeal seta nude, AL > AM ..... *guatemalensis* Brennan
   Galeal seta branched, AM > or = AL ..... *pedregalensis* (Hoffmann)
8. (4) Palpal genual seta nude ..... *scitula* n.sp.
   Palpal genual seta branched ..... 9
9. Number and arrangement of posterior sternal and coxal III setae abnormal ..... *anomala* (Hoffmann)
   Number and arrangement of these setae normal ..... 10
10. AM > AL, galeal seta usually branched, tarsala I considerably longer than tarsala II ..... *diazi* (Hoffmann)
    AL > AM, galeal seta nude, tarsala I < or = tarsala II ..... 11
11. Length of tarsala I < tarsala II, setules of sensillae strong and conspicuous, microgenuala I and microtibiala I short ..... *hungerfordi* Lipovsky
    Length of tarsala I = tarsala II, setules of sensillae weak and nearly imperceptible, microgenuala I and microtibiala I long ..... *audyi* n.sp.


## SUMMARY

The genus *Pseudoschöngastia* Lipovsky is redescribed and the species listed. The generic status of the anomalous *P. novita* Audy and Womersley is questioned. *Ascoschöngastia anomala* Hoffmann and *A. pedregalensis* Hoffmann are transferred to *Pseudoschöngastia.* Four new species are described and figured: *P. aberrans* off

*Peromyscus, P. hypopsia* off *Baiomys, P. scitula* off *Liomys,* all from Chiapas, Mexico, and *P. audyi* off *Peromyscus* from Sacatepequez, Guatemala. A key to species listed is given.

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(continued from p. 404)

FIGURE 2. Carapace of *C. sapidus* showing holes (indicated by arrows).

## A NOTE ON THE DISTRIBUTION OF THE TICK *AMBLYOMMA IMITATOR* KOHLS, 1958

In his original description of *Amblyomma imitator,* Kohls (1958, J. Parasit. **44:** 430–433) reported finding specimens of his new species among ticks labeled *Amblyomma cajennense* (Fabricius, 1787) in several collections at the Rocky Mountain Laboratory, Hamilton, Montana, that had been taken from a wide variety of domestic and wild animals and man. Since the distribution of this new species was limited to southern Texas and Mexico, it occurred to the present writer that a comparable study of ticks in the Helminthological Collection of the United States National Museum might yield further specimens of *A. imitator* from other geographical regions, thereby extending its known range.

Re-examination of 58 lots of ticks labeled *A. cajennense* or *Amblyomma* sp. revealed 2 lots which included specimens identified as *A. imitator* by the writer. U.S.N.M. Helm. Coll. No. 15663 was collected from *Bos taurus* in Honduras, Central America, and consisted of 4 females and 6 males. One female and 1 male were identified as *A. imitator,* and were recatalogued as U.S.N.M. Helm. Coll. No. 38858. The second lot, U.S.N.M. Helm. Coll. No. 31539, was composed of 3 females that had been collected from *Equus caballus* at Harlingen, Texas. These ticks were all identified as *A. imitator.*

The female specimens of *A. imitator* were easily differentiated from those of *A. cajennense* by using the distinguishing characteristics described by Kohls; namely, the absence of chitinous tubercles at the posterointernal angle of the festoons and certain morphological details of the external genitalia. The male of this species was also distinguished from that of *A. cajennense* by its smaller size and the ornamentation noted by Kohls.

Finding *A. imitator* among ticks collected in Honduras extends its range to Central America.—WILLARD W. BECKLUND, *Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.*

# THE EFFECT OF WEATHER ON THE DEVELOPMENT AND SURVIVAL OF THE FREE-LIVING STAGES OF *NEMATODIRUS SPATHIGER* (NEMATODA: TRICHOSTRONGYLIDAE)

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Interest has been generated in recent years in *Nematodirus spp.* of sheep, since it has been demonstrated that species of this genus are pathogenic under both natural and experimental conditions. Kates and Turner (1953) and Seghetti and Senger (1958) have demonstrated experimentally that large numbers of *Nematodirus* cause diarrhea, weight losses and even death in young lambs. Kingsbury (1953) discussed the natural outbreaks of *Nematodirus* disease in lambs in Britain, and reported finding nearly pure infections with an average worm burden of 11,626 in 19 lambs. He noted that the disease was seen in lambs on older pasture and not in lambs on "first-year leys." Additional ecologic work has shown that, in Britain, nematodiriasis is a lamb-to-lamb disease in which the free-living stages of the parasite survive on the pasture from one growing season to the next. (Baxter, 1957; Gibson, 1958; Stamp, et al, 1955; Spedding et al, 1958; and Thomas and Stevens, 1956).

Three species of *Nematodirus* in sheep are known to cause disease, *N. spathiger* (Railliet, 1896), *N. filicollis* (Rudolphi, 1802), and *N. battus* (Crofton and Thomas, 1951). *N. spathiger* has been the cause of parasitosis in South Africa (LaPage, 1956) and in the United States. *N. battus* and *N. filicollis* are the cause of nematodiriasis in Britain (Kingsbury, 1953); the former has not yet been reported as occurring elsewhere.

During the 1940's diarrheas and deaths were seen in lambs in Montana, apparently resulting from heavy infestations of *Nematodirus spathiger*. Such outbreaks of intestinal parasitism have been observed in fall and early winter. Since no bionomic studies on *N. spathiger* have been reported from an area having climatic extremes such as are found in western Montana, a series of experiments was undertaken both in the laboratory and on an experimental outdoor plot in order to determine more precisely the pattern of development and survival of this parasite.

## MATERIALS AND METHODS

### *Climate*

This work was carried out near Bozeman, Montana, which is situated in a moutain valley at an elevation of 4900 feet. The average annual rainfall is 17.6 inches with a little more than half falling during the first 6 months of the year. Average temperatures are 24.9° F for the period January through March; 50.4° F for April through June; 62.1° F for July through Sep-

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tember; and 30.6° F for October through December. Over the years from 1925 to 1954 the average year had 5 days with a maximum of 90° or higher; 50 days with a maximum of 32° or lower. The temperature fell to freezing or below on 181 days and below 0° on 19 days. The mean daily difference between maximum and minimum temperatures was 23.6° F, an average consistent with both summer and winter daily fluctuations (Dightman, 1954). The growing season is generally 115 days, May 24 to September 15 (Climate and Man—Yearbook of Agriculture, 1941). Sunlight is intense particularly during the summer and fall; winter days are generally partly cloudy or clear. The relative humidity is low and evaporation high.

*Weather data*

Weather data were recorded at the experimental plot. A hygrothermograph constantly recorded the air temperature and relative humidity. A recording thermograph gave temperatures at the soil surface, a half inch below the surface and 1 inch below the surface. Some of the later soil surface temperatures were obtained from the weather station of Montana State College. Figure 4 contains maximum, minimum and mean soil surface temperatures for the period of observation.

Precipitation records were obtained from the records of the Montana State College weather station located about a half mile from the experimental plot.

*Experimental Plot*

Experiments were carried out on a small enclosure adjacent to a sheep pasture. The drainage was such that there was little possibility of contaminating the plot. Any grasses on the plot were clipped close to the ground whenever necessary.

Ova and larvae were exposed to 2 different microhabitats on this plot. Half of the samples were placed on the ground in the open. The other half were placed under a wooden frame covered with a single layer of burlap. This frame was raised 8 inches off the ground, and provided rather dense shade, but allowed for circulation of air. Except after extended dry periods, the soil under the cover retained some moisture.

*Development of ova*

In testing development of ova, formed sheep pellets containing 200 to 800 *N. spathiger* ova per gram were exposed to the weather on the experimental plot. In the early tests, pellets were placed on a 6 cm Seitz filter pad (fig. 1, A and B) which was ringed with half-inch cellophane tape. However, pellets disappeared from the pads in a short time, and it was found that annelid worms were removing them. When the fecal pellets were tied in cheesecloth bags and attached to the pad, the earthworms fed on the pellets without removing them. Ultimately a piece of metal fly screen was fastened to the ground and the pads, in turn, placed on it. No obvious interference by invertebrates was subsequently observed.

In preliminary experiments, development and survival was given in terms of recovered ova capable of developing to infective larvae under favorable conditions. Since there was no measure of the total number of the original ova which were capable of developing, a more elaborate series of experiments were designed and carried out.

Ten well distributed samples were taken from any single gallon jar of fecal pellets, and the ova counted by coverslip flotation in sugar. Ova were placed in water in petri dishes and allowed to develop at 30° C thereby obtaining a percentage hatch to use as a reference point. Ten grams of feces were tied up in small cheesecloth bags and placed out on the experimental plot. Half of the samples were placed in the open, and half under the burlap cover. Groups of 50 samples were placed out on January 15, May 1, and September 1, 1957, and a short test comprising 20 samples was started in July 1957. Duplicate samples were picked up twice monthly, except during the late winter when it was impossible to retrieve the samples because of deep snow. There was a 2-month overlap between any 2 experiments. Thus, pellets which had been exposed to the weather for 5 months could be compared to those only out for 1 month. Ova from these samples were examined microscopically and the numbers in various stages of development were counted. The following stages of development were noted: segmented, morula, tadpole, larva, hatched larva and empty shell, (approximately as illustrated by Kates and Turner (1955) in plate 1, figs. 1, 2, 3, 5, and 7, respectively). Filter pads were baermannized to obtain any larvae which might have hatched and left the fecal pellets, but no larvae were ever found on the pads.

*Survival of larvae*

Larvae used for experimental exposure were obtained by the method of Seghetti (1955). A volume of larval suspension calculated to contain 1,000 larvae was placed on each Seitz filter pad ringed with cellophane tape. The pads were dated and attached to the ground with a short

nail (fig. 1, B and C). Pads were put out and picked up semi-monthly at the start of the experiment and monthly after preliminary information had been obtained, except during the late winter months when snow cover was generally too deep. Larvae were recovered from the pads

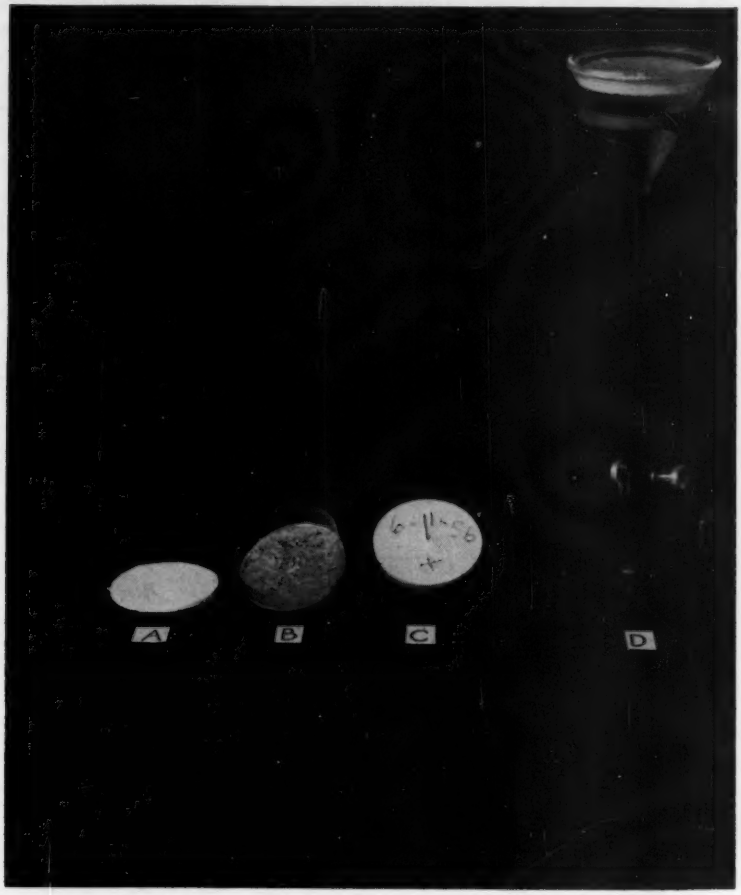


FIGURE 1. Asbestos filter pads and Baermann apparatus. A. Seitz asbestos filter pad, 6 cm in diameter. B. Pad ringed with cellophane tape and containing suspension of larvae. C. Under-surface of pad showing date and nail for attachment to ground. D. Small funnel used for baermannizing pads.

by baermannizing in long-stemmed glass funnels (fig. 1, D). Of larvae placed on such pads, 85 to 95 percent can be recovered from the pad after drying.

No attempt was made to distinguish between live and dead larvae. It was assumed that any larvae active enough to leave the pad and fall to the bottom of the funnel were alive and they were therefore counted.

## RESULTS

### *Development of ova*

Figures 2 and 3 represent data obtained in an experiment on development of ova starting January 11, 1957, and terminating December 15, 1957. The figures show

the percentage of undeveloped ova, (segmented), developing ova (morula, tadpole, 1st and 2nd stage larvae), empty shells and infective larvae. It has not been possible to determine the exact status of the empty shells, that is, whether they represent dead ova or ova from which the infective larvae have escaped. Since the number of recovered infective larvae did not exceed 14.5 percent, and was usually much lower, the counting of the empty shell was considered unimportant.

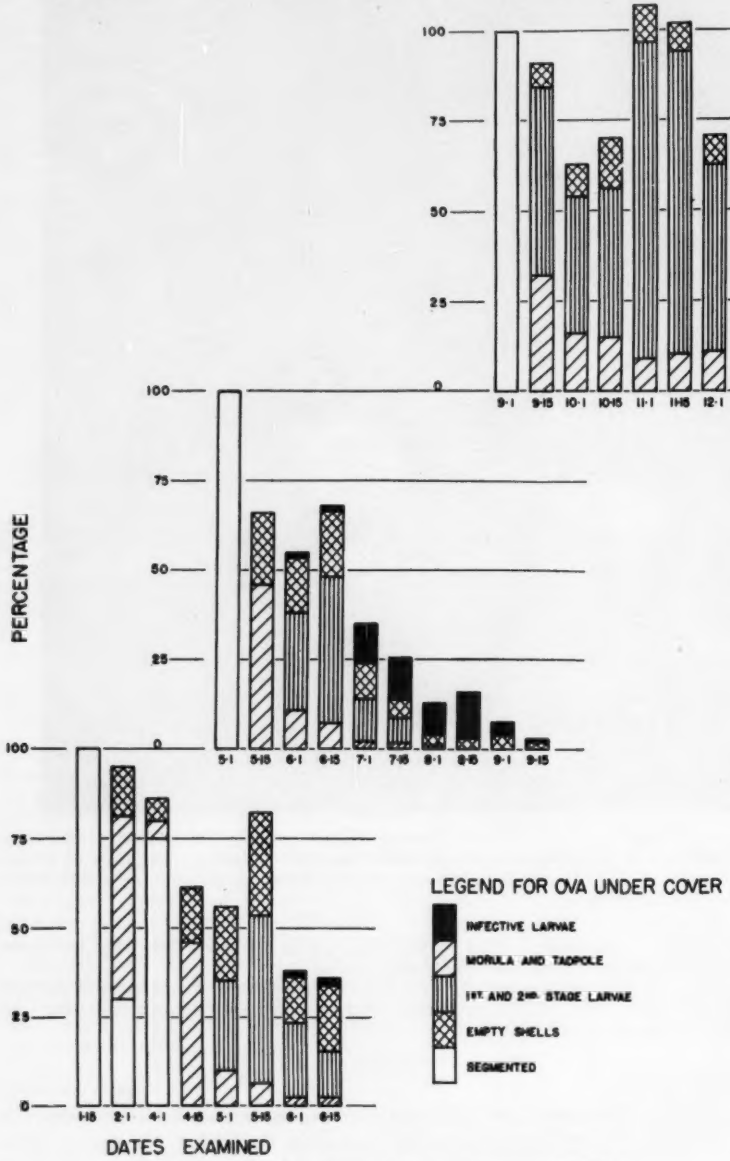


FIGURE 2. Development of ova under cover (1957).

The data obtained on development and survival of ova either protected by the cover or exposed in the open differ only slightly. It has been our observation in other experiments of this type that ova placed out in November and December do not develop to any extent until the following spring. The ova set out in January, for

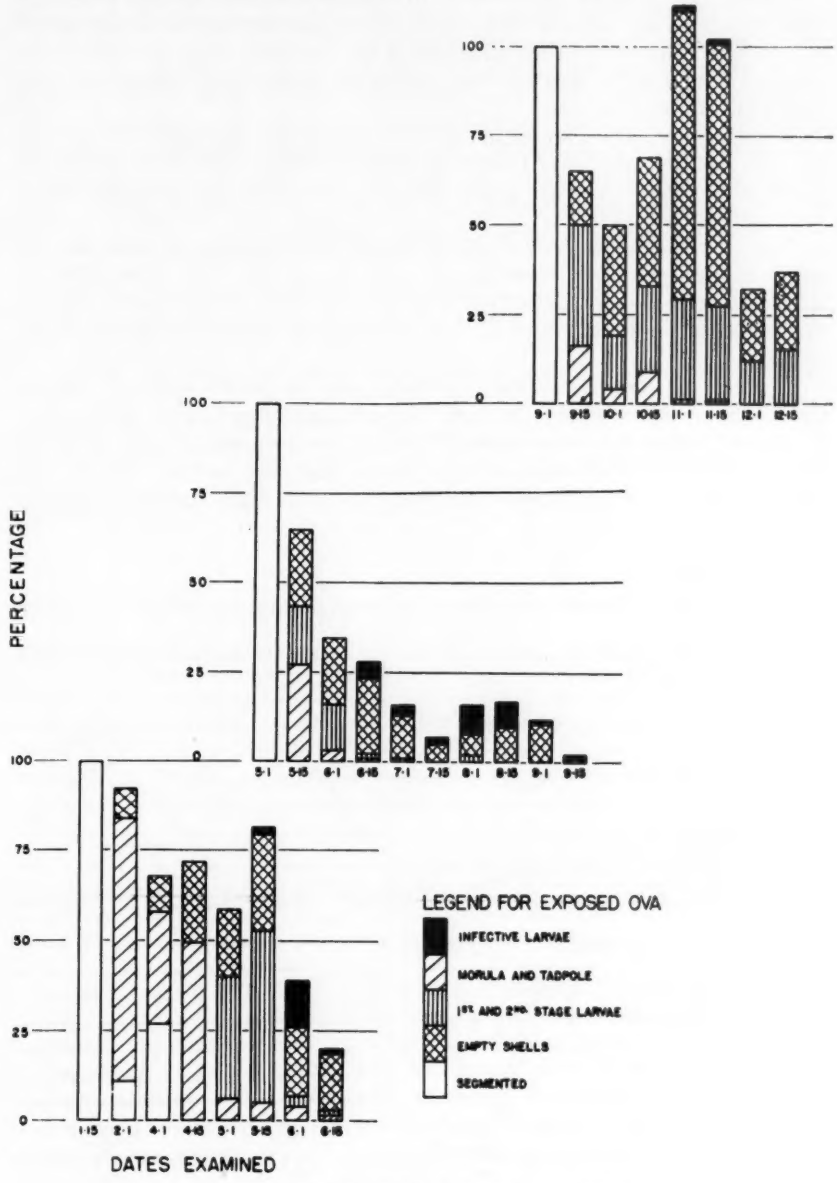


FIGURE 3. Development of exposed ova (1957).

example, developed rather slowly until about April. More rapid development took place at this time in exposed ova than in those protected by the cover. Rapid development and rapid death, in the later months as shown by the sharp decline in segmented ova, takes place from May to October.

During May, June and July there is little difference between the rate of development of exposed and protected ova. In a short experiment starting in July, development took place at about the same rate in the open and under the cover. The recovery of those in the open was lower during the developmental period indicating more rapid death of the ova.

In the 2 groups of ova set out in September, the development proceeded at about the same rate. No ova hatched under the cover, but about 1 percent hatched in the open. Little death had occurred in either group by the termination of the experiment on December 15.

Figures 2 and 3 show a slow development and low survival in the colder weather and rapid development with short survival in the hot months. The cover provides a somewhat cooler environment and protection from the sun. The net effect is retardation of development and longer survival as compared to the exposed free-living stages.

The data obtained in the 1957 experiment agree in general with our previous observations on development. In April, May and June, there is some development. In July and August the ova or larvae are killed in the open, but somewhat protected by the cover. Partial development takes place in September and October, but usually stops by November. Development proceeds rapidly again starting in April or early May.

*Larval Survival*

Figure 4 summarizes the data obtained on larval survival and includes maximum,

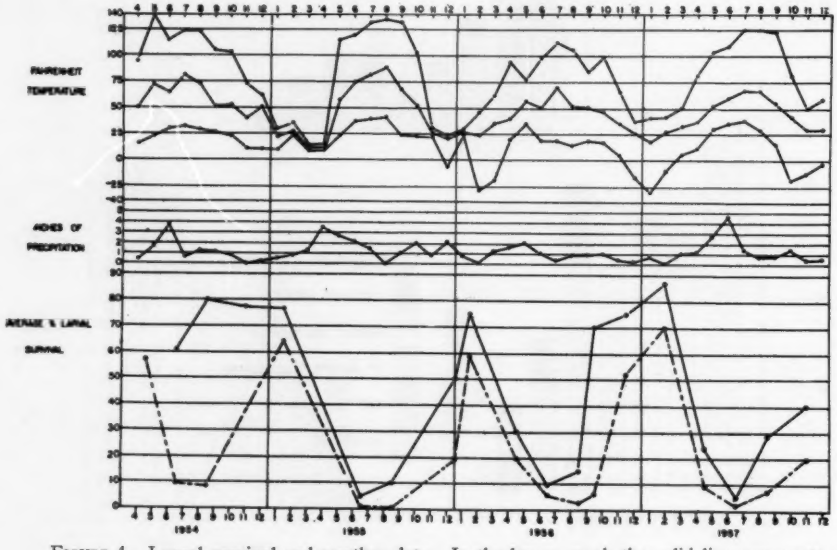


FIGURE 4. Larval survival and weather data. In the lower graph the solid line represents

percentage survival of larvae protected by the cover; the broken line, percentage survival of larvae in the open. The upper graph gives monthly maximum, minimum and mean soil surface temperatures.

minimum and mean monthly soil surface temperatures. Monthly precipitation is recorded in inches. The data obtained on larval survival are plotted separately for the larvae placed in the open and those placed under the cover. Each point on the graph represents the average of the number of larvae surviving for 1 and 2 months. This would comprise 4000 larvae. Averages for April, May and June survival were generally obtained from material set out in the previous winter, since it was impossible to set out or pick up material sometimes from January to May. Table I shows the reason for simplifying the data in this manner, and the necessity for taking averages over a period as long as 5 months. In 1955 larvae could not be set out in April or May, although it was possible to pick up some in May. During the winter of 1955–56 larvae were set out in November, and they were not picked up again until April, 5 months later.

The data show infective larvae survive well during the cold part of the year, and poorly during the hot part of the year. In table I, for example, 22 percent of the

TABLE I. *Percentage of* N. spathiger *infective larvae surviving through various seasons*

| | Jan. | Feb. | March | May | 1955 June | July | Aug. | Sept. | Oct. | Nov. | 1956 April |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Exposed | 100 | 70 | 61 | 8 | 0 | | | | | | |
| | | 100 | 61 | 33 | 9 | | | | | | |
| | | | 100 | 4 | | | | | | | |
| | | | | | 100 | < 1 | < 1 | 0 | | | |
| | | | | | | 100 | 5 | 1 | 0 | | |
| | | | | | | | 100 | 2 | 0 | | |
| | | | | | | | | 100 | 1 | < 1 | 0 |
| | | | | | | | | | 100 | — | 20 |
| | | | | | | | | | | 100 | 22 |
| Covered | 100 | 91 | 68 | 34 | 10 | 0 | | | | | |
| | | 100 | 50 | 32 | 52 | — | 0 | | | | |
| | | | 100 | 15 | 36 | | | | | | |
| | | | | | 100 | 10 | 3 | 13 | < 1 | < 1 | |
| | | | | | | 100 | 4 | 18 | 20 | 41 | |
| | | | | | | | 100 | 12 | 3 | 4 | 0 |
| | | | | | | | | 100 | 11 | 15 | 0 |
| | | | | | | | | | 100 | 20 | 30 |
| | | | | | | | | | | 100 | 46 |

Dashes indicate no sample picked up.

larvae set out in the open and 46 percent set out under the cover in November, 1955, survived until the following April. On the other hand, fewer than 1 percent of the larvae set out in the open in June were recovered in July. Of larvae set out under the cover at the same time, 10 percent survived one month. Figure 4 shows this type of pattern over nearly a 4-year period.

## DISCUSSION

The resistance of members of the genus *Nematodirus* to environmental conditions has been well established. Kates (1950) reviewed the literature on the survival of free-living stages and concluded that among the nematodes of sheep the free-living stages of *Nematodirus* are the most resistant to the entire annual complex of climatic conditions. His experiments on natural acquisition of infestations with *Nematodirus* show that fewer worms were picked up in the summer than in fall. Some pastures seeded with ova in fall allowed sheep to acquire fewer numbers of parasites in fall than they did the following spring.

Turner's (1953) experiments concerned recovery of *Nematodirus spathiger* larvae from pasture, and the effect of temperature and moisture on free-living stages. He showed not only survival over the winter, but that 70 percent of larvae were recovered from the grass, as opposed to the humus and the subsurface layer, in the winter–spring period, and 20 percent recovered from the grass in the summer–fall period. Poole (1956) showed that larvae of *Nematodirus filicollis* can survive temperatures as low as – 65° C, and that repeated freezing at – 6.5° C and thawing had little effect on them. Our experiments reported here confirm the resistance of *Nematodirus* ova and larvae to adverse environmental conditions.

In Britain, Thomas and Stevens (1956) observed a sharp increase in the numbers of *Nematodirus battus* and *N. filicollis* during the 4th week of April on a pasture naturally seeded with ova the previous summer. Spedding et al (1958) noted an earlier rise in numbers of larvae on the grass, but confirmed the concept proposed by Stamp, et al (1955) of a lamb-to-lamb transfer. This increase in numbers of larvae on the herbage is apparently a result of both development of ova in the spring and migration of infective larvae up onto the grass.

Our experiments on development in nature agree in principle with other observations. While we did observe development to infectivity in the fall, most of the ova did not reach the infective stage until the following spring. In experiments carried out in southeastern Montana, an area having a fall climate much like the Bozeman area, worm-free lambs became infected with *Nematodirus* during any season, but larger numbers were acquired in fall and spring (Seghetti, 1948). Compared to observations made in areas having a milder climate than Montana, the main difference we observed is only fractional development and survival of free-living stages of Nematodirus through the growing season. Of those ova deposited on the pasture in late June, July, August and early September, only a small percentage will reach infectivity if they are exposed to the sun. According to Gibson's (1958) studies on *N. battus* and *N. filicollis,* ova deposited on the pasture in spring generally reach the infective stage the following spring. Under the climatic conditions of Montana, *N. spathiger* free-living stages survive only from fall to spring.

It is inferred from our laboratory and field observations that sunlight itself and high temperatures are the agents most detrimental to the free-living stages of *N. spathiger*. On a clear day in May, washed ova were placed in tap water having a depth of about 1 centimeter and set in the sun. The temperature of the water was maintained below 30° C (83° F), a level known to be harmless to *N. spathiger*. It was found that the ova were killed by less than 8 hours exposure to sunlight under these conditions. In other experiments infective larvae were killed by the direct rays of the sun in 4 to 5 days, but they survied repeated drying and rehydration. Autumn is typically a time of mild weather in this area with almost continual sunshine, but lower temperatures, and is a time of year when larval survival again begins to increase.

Examination of figure 4 shows that moisture, expressed as precipitation, bears no relation to the length of larval survival. It is suggested, however, that ground-surface temperatures and direct sunlight do bear a relation to the length of survival. The mean maximum soil and surface temperatures reach a peak of 110° F to 130° F at a time when survival is low. As the soil-surface temperatures begin to fall and the angle of the sun decreases, the time of larval survival begins to increase.

SUMMARY

1. A 4-year study is reported on the effect of southwestern Montana weather on the development and survival of *Nematodirus spathiger* of sheep.

2. Development of ova occurred during nearly any season of the year except the coldest months of the winter. Most rapid development took place from April through September. Development was slow and few embryos developed to the infective stage from October through March.

3. Infective larvae survived longest in the cool or cold seasons. At least 10 percent of the larvae could survive the winter.

4. The complex of relatively slow development and long survival of the free-living stages of *N. spathiger* indicates that the largest numbers of infective larvae occur on pasture in spring.

5. It is inferred from the data that the agents most detrimental to the free-living stages of *N. spathiger* are sunlight and a high soil-surface temperature.

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## RESEARCH NOTE

### THE SEASONAL INCIDENCE OF INFECTION OF THE SNAIL *NASSARIUS OBSOLETUS* (SAY) WITH LARVAL TREMATODES

Seasonal incidence of infection of *Nassarius obsoletus* (Say) with larval trematodes was first reported by Miller and Northup (1926, Biol. Bull. **50:** 490–509). Because the presence of a dermatitis-producing avian schistosome larva, *Austrobilharzia variglandis* (Miller and Northup, 1926) Penner, 1953, in *N. obsoletus* has public health significance, investigations of the seasonal variations of this particular parasite infection and its host-parasite relations recently have been given greater impetus on both the east coast (Penner, 1953, J. Parasit. **39**(supp.): 20; Sindermann, 1956, J. Parasit. **42**(supp.): 27; Stunkard and Hinchcliffe, 1952, J. Parasit. **38:** 248–265) and the west coast (Grodhaus and Keh, 1958, J. Parasit. **44:** 633–638) of the United States. It was, therefore, thought that this report of work undertaken in 1951 and 1952 would be of interest.

Collections of *N. obsoletus* were made monthly from October 1951 to June 1952 from the mudflats of Greenwich Bay several miles east of Apponaug, Rhode Island. An effort was made to obtain random samplings from an area covering approximately 1000 yards of shore line. In addition, from March 1952 to June 1952 similar collections were made from a tide pool in the high-tide zone of the same area. The snails were isolated in the laboratory and observed for several days to determine those with emerging infections. Ultimately all the snails, those with and those without emerging cercariae, were crushed.

Shell measurements were made on a large portion of the snails. Infected individuals tended to be larger than the uninfected. The infection incidence in each of 4 categories of size groups was as follows: 10–12 mm, 36 examined, 2 infected (5.5 percent); 13–15 mm, 1473 examined, 110 infected (7.5 percent); 16–18 mm, 1667 examined, 315 infected (19.0 percent); 19–23 mm, 65 examined, 26 infected (40.0 percent). The mean size of the snails measured was greatest in late spring and autumn, and least in December and January.

A total of 6717 snails were examined, and 1445 were found infected with 1 or more of the 8 species of larval trematodes known to infect *N. obsoletus* on the east coast of the United States. Infection data are summarized in table I.

The incidence of infection was low from October to early March. During this period, larvae of *Zoogonus lasius* (Leidy, 1891) Stunkard, 1940, and *Himasthla quissetensis* (Miller and Northup, 1926) Stunkard, 1938, were responsible for the majority of infections, whereas only 1 or 2 *A. variglandis* infections were found each month. Larvae of *Lepocreadium setiferoides* (Miller and Northup, 1926) Martin, 1938, *Stephanostomum tenue* Martin, 1939, *Gynaecotyla adunca* (Linton, 1905) Rankin, 1940, and *Cercaria dipterocerca* Miller and Northup, 1926, occurred sporadically throughout the period of the investigation. Immature sporocysts of *Cercaria nassa* Martin, 1945, were found in a 1 to 2 percent incidence from November to May. In the June collection, 4 snails were found with mature *C. nassa* sporocysts from which the typical strigeid cercariae were observed emerging.

The number of infected snails rose sharply in the March 21st collection with *Z. lasius, H. quissetensis,* and *A. variglandis* as the most prevalent forms. The tide-pool snails, which were first collected on the March 21st date, showed an even higher infection incidence than those collected in the low-tide zones.

In April and May the over-all infection incidence tapered off with the tide-pool snails retaining the higher incidence. It is of considerable interest that infections all but disappeared in the low-tide zone by June and, aside from *Z. lasius* and *H. quissetensis,* were markedly reduced in the tide-pool snails as well. The incidence of *A. variglandis* dropped precipitously at both collection sites in late spring. It may be significant that there were many dead snails in the June collection.

These data suggest that infection of the snails occurs in early spring when the definitive hosts are migrating (birds northward, eels shoreward). The peak of infection with *A. variglandis* in the Autumn as reported by Sindermann (1956, loc. cit) can possibly be accounted for similarly by the southward migratory trek of the avian hosts. The parallel infection rates of snails collected from below the mean low-tide level and those collected from the tide-pool suggest that infection fluctuations in *N. obsoletus* are not a result of differential snail migrations.

(The author wishes to acknowledge the guidance of Dr. Lawrence R. Penner in this work.)

*(continued on p. 456)*

# STUDIES ON FECAL TRANSMISSION OF *TRICHINELLA SPIRALIS**

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Infections of *Trichinella spiralis* were found in representatives of 8 species of wildlife in Iowa (Zimmermann, Hubbard and Biester, 1959); viz., the rat, mink, fox, striped skunk, spotted skunk, opossum, raccoon and coyote. Since numerous species of wildlife had been found to be infected with *T. spiralis*, investigations were made to determine a possible relationship between infected wildlife and grain-fed swine in the trichiniasis cycle. Two modes of transmission considered were (1) consumption of infected wildlife carcasses by swine, and (2) fecal transmission from infected animals to susceptible animals. Rats and mice may occasionally be eaten by swine, while other forms of wildlife are less available. Fecal transmission experiments were conducted since many species of wildlife are nocturnal in their habits and may contaminate hog lots and pastures with their feces. Swine feed may also contain rat and mouse feces. Spindler (1953) transmitted trichinae to swine by feeding them feces of dogs, cats, swine and albino rats that had consumed trichinous flesh. Transmission was obtained during both the maturation period of intestinal trichinae (days 1–4) and during the adult stages (days 4–28). Olsen and Robinson (1958) transmitted trichinae by feces from mouse to mouse, rat to rat, mouse to pig, and rat to pig. The greatest number of trichina larvae and cysts were eliminated during the first 24 hours after infection. Previously infected animals eliminated a higher percentage of the infective dose than those animals infected only once. McCoy (1938) fed *T. spiralis* larvae to immune rats and found that numerous larvae appeared in the feces, the majority within 18 hours after infection. These larvae were alive and developed normally when fed to nonimmune rats.

## MATERIALS AND METHODS

Fox cubs and albino rats, herein designated as donor animals, were experimentally infected with larvae of *T. spiralis* and placed in cages above the feed troughs or dishes of swine, fox cubs, or albino rats designated as recipient animals (Fig. 1). All feces from the donor animals fell into the troughs or dishes below. The factors considered were (1) time interval between infection of donor animal and placement of donor in cages above recipient, (2) status of donors as regards immunity to trichinae, and (3) size of infecting dose.

The method of infecting the donors varied: the foxes were fed flesh of trichinous rats; the albino rats were inoculated with a liquid suspension of active larvae which had been digested free of host tissue and administered by means of a stomach tube. The size of the infecting doses given the foxes was not calculated except in 1 series in which approximately 40,000 larval trichinae were fed to establish initial infections. An estimated 80,000 larvae constituted the reinfecting dose. All other foxes were given massive infections. The infective dose for the rats varied from 4000–10,000 larvae.

After the donor animals were infected, they were placed in holding cages away from the pens of the recipients. After definite periods of time, herein referred to as the postinfection interval, the animals were removed from the holding cage and placed in cages above the feed troughs or dishes of the recipients. The postinfection intervals used in these experiments were

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4 hours, 1 day, 4 days, and 6 days.

Donor animals were kept over recipient's cages or pens for 28 days. After an additional 28 days the recipients were killed, and 45 gm samples of selected tissues were examined by the artificial digestion-Baermann method. The tissues from which these samples were procured were the diaphragm in the case of swine; diaphragm, tongue, and posterior leg muscles from foxes; in the case of rats the skinned and eviscerated carcass was used.

Immunization of donor animals was by oral administration of larval trichinae at intervals of 28 days or more. The total number of infective doses are indicated in the text by the terms 1st, 2nd, or 3rd infection.

In addition to actual transmission studies, fecal droppings were collected from experimentally infected fox cubs and albino rats at various time intervals and examined by the Baermann technique. Determinations were made of the percentage of larvae which passed through the digestive tract during various time intervals.

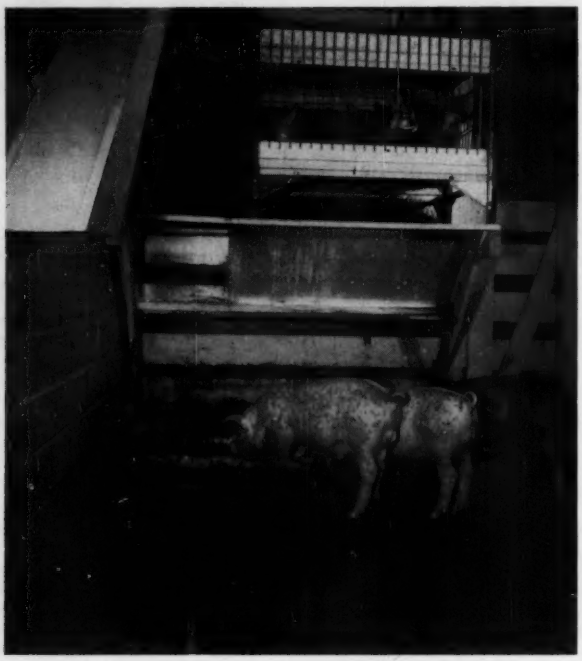

FIGURE 1. Experimental cages and pen, back view.

Swine known to be free of trichinae were used as recipient animals in the trials. These swine were obtained from a herd maintained for experimental purposes at the Veterinary Medical Research Institute, Iowa State College. This herd was developed from swine taken by hysterotomy, raised in isolation, and maintained in quarantine. Rodent control is maintained on a continuous basis. Albino rats also known to be free of trichinae were obtained from a closed colony maintained for experimental purposes. Wild fox cubs were obtained from dens when approximately 6–10 weeks of age. Although this did not preclude prior infection, only 1 of 79 other fox cubs which were obtained from this same area in field studies during the past several years was found to be infected with *T. spiralis*.

## RESULTS

### *Fox to Pig Transmission*

Twenty trials involving transmission from 93 foxes to 48 pigs were conducted. Experimental groups generally consisted of 4–6 fox cubs and 2–3 pigs. Transmis-

sion occurred most readily when 4 hours had elapsed between oral infection and placement of foxes in cages over swine feed troughs. Foxes infected for the first time and placed over pigs 4 hours later transmitted trichinae to 12 of 13 pigs. The number of larvae recovered from 45 gm samples of diaphragm muscle tissue taken from recipient swine varied to the extent that 8 of the 12 counts were less than 150, while 4 were more than 12,000, the maximum being 68,000.

Reinfection of the donor foxes 4 weeks after the initial infection increased the degree of transmission. All 9 of the recipient pigs became infected in tests using reinfected donors and a 4-hour postinfection interval. The smallest number of larvae obtained from diaphragm samples was 420, the maximum 58,000. Five of the 9 counts were greater than 20,000.

Transmission was markedly decreased when a 1-day postinfection interval was used. In 4 trials using 1st-infection foxes, fecal transmission occurred in 3 of 9 pigs. Trichinae counts made on samples from the diaphragms of these infected pigs were 2, 4 and 9 larvae, respectively. One test with 2nd-infection foxes, 1-day postinfection interval, was negative.

In 2 tests using a 4-day postinfection interval with 1st-infection foxes, 1 of 5 pigs became infected. A larval count of 12 was obtained. Similar results were obtained when 2nd-infection foxes were employed. One of 5 pigs became infected, 22 larvae being recovered from the 45 gm sample of diaphragm. No transmission was obtained in 2 tests using a 6-day postinfection interval.

A high mortality occurred among the donor foxes. One of 13 of these animals died when a dose level of 40,000 larvae was used. The number of larvae fed the other fox cubs for the initial infection was sufficient to cause death of 21 of 50, most deaths occurring between days 2 and 7. Two foxes died on the 17th day as a result of a 2nd infection.

*Rat to Pig Transmission*

Ten rat to pig transmission trials were carried out, using 21 rats and 18 pigs with experimental groups generally consisting of 2 rats and 2 pigs. Transmission became established only when a 4-hour interval elapsed between infection of the rats and placement over the recipient pigs. Rats with various degrees of immunity were used in a series utilizing a 4-hour postinfection interval. Neither of 2 pigs became infected from the ingestion of feces from 1st-infection rats. One of 2 pigs was infected from 2nd-infection rat fecal material, while both pigs were infected from the feces of 3rd-infection rats. The infections in the swine were mild; the number of larvae found in the 45 gm porcine diaphragm samples were 56, 39 and 12, respectively.

No transmission was obtained in rat to pig transmission trials using 1st-infection rats with 1- and 6-day postinfection intervals and 3rd-infection rats with 4-hour, 1-day and 4-day postinfection intervals.

Five donor rats died as a result of the infections. Three deaths were observed following the initial infection and 1 each following the 2nd and 3rd infections.

One rat-to-fox and 2 rat-to-rat trials were conducted, but transmission did not occur. Nonimmune donor animals were used with a 1-day postinfection interval.

*Fox Fecal Studies (Baermann technique)*

Five foxes were given an initial infective dose of approximately 40,000 *T. spiralis*. Larval trichinae were recovered from the feces of all animals, the number

ranging from 2 to 337 during the 4-day postinfection period. A total of 690 larvae was egested by the 5 animals, of which 652 (94.5%) were passed in the first 24-hour period after ingestion of the trichinous rat meat. Thirty larvae were recovered during the second 24-hour period, 8 during the 3rd, and none during the 4th. The 5 foxes were then placed over a pen containing 3 pigs for the next 28 days. Twelve larvae were recovered from the 45 gm sample of the diaphragm from the 1 pig which became infected.

Each of the fox cubs was reinfected with approximately 80,000 trichina larvae 5 weeks after the 1st infection. The number of larvae recovered from the feces ranged from 167 to 9983. More than 99% of those recovered were passed in the first 24-hour period after infection. The maximum count, 9983, represented approximately 12.5% of the infective dose. After fecal collection on the 4th day, the foxes were placed over a pen containing 3 pigs. Transmission did not occur.

TABLE I. *Recovery of* T. spiralis *larvae from albino rat feces*

| Infection | Dosage | No. of rats | Larvae recovered |
|---|---|---|---|
| First | 4000 | 6 | 0 ; 0 ; 14 ; 19 ; 117 ; 187 |
| | 8000 | 2 | 55 ; 130 |
| Second | 4000 | 3 | 24 ; 38 ; 56 |
| | 8000 | 1 | 255 |
| Third | 4000 | 2 | 0 ; 17 |
| Fourth | 10000 | 2 | 596 ; 923 |
| Fifth | 8000 | 2 | 97 ; 314 |
| Sixth | 8000 | 2 | 171 ; 1693 |

*Rat Fecal Studies (Baermann technique)*

The number of larvae recovered from rat feces by the Baermann technique varied with the number of immunizing doses (Table 1). Larvae were egested in the feces of 17 of 20 rats during the 4-day postinfection period. Two of the 3 rats which did not pass trichinae had received an initial infection of 4000 larvae, while the 3rd rat had received a 3rd infection of 4000. Larvae were obtained from droppings as early as 4 hours after infection. Over 95% of the larvae recovered were found in the first 24-hour postinfection period. The percentage of the administered dose recovered in the feces varied. The maximum, 21.2%, was recovered from the feces of an albino rat which received a 6th infection.

## DISCUSSION

The results of the investigations herein reported indicate that experimental transmission of *T. spiralis* to swine can be obtained through the medium of feces of infected foxes. It emphasizes the possible importance of wildlife reservoirs in the spread of trichiniasis. The time interval between infection of the donor and passage of feces is a prime limiting factor. In these studies, 95% or more of the larvae recovered from fox and rat feces by the Baermann technique were in feces eliminated during the first 24-hour period after infection. Therefore, any feces passed within this 24-hour period should be considered as a potential source of infection of susceptible hosts that consume these feces. This finding was corroborated by actual transmission studies. In the trials, 21 of 22 pigs became infected by consuming fox feces when the interval between infection and placement of fox cubs over the swine pens was 4 hours. When the postinfection interval was extended to 24 hours, only 3 of 12 pigs became infected. Environmental factors such as temperature and

desiccation may tend to limit the viability of trichina larvae in feces. Olsen and Robinson (1958) found that infectivity was decreased by 50% or more when rat and mouse feces were dried for a period of 4–8 hours.

A second factor affecting fecal transmission is previously acquired immunity of the donor animals. Heavier infections were obtained in transmission experiments using immune donor animals. A larger percentage of the administered dose was found in the feces of immune than in nonimmune animals as indicated by Baermann studies.

A third factor affecting transmission is the number of viable trichinae ingested by donors. Larval counts made on the feces of infected rats showed that the number of larvae recovered was directly proportionate to the size of the infective dose.

In these studies transmission was primarily accomplished with excysted trichina larvae. The larvae had been digested free of host tissue, but had not become established in the intestine of the donor. Some encysted larvae may have been passed in the feces, but were not demonstrable by the Baermann technique.

The degree of transmission was greater when foxes were used as donors than when albino rats were used. Normal fecal transmission requires the infective larvae to undergo two digestive processes, one in the donor and the other in the recipient animal. The artificial digestion method was used to free larvae from host tissue prior to administering them to albino rats. This extra digestive process may be a factor in the decreased frequency of transmission when albino rats served as donors. Investigations are in progress which will eliminate the in vitro digestive process. Another factor may be donor-recipient proportions used. In fox-to-swine transmission studies 2 fox donors were generally used for each swine recipient, while for the rat-to-swine studies the proportion was 1 rat to 1 pig. If the latter proportion were increased to equal that in the fox-to-pig studies, a higher rate of transmission would probably be obtained. The possible role of raccoons, opossums, hawks and other wildlife species in perpetuation of trichiniasis through fecal transmission is also being studied.

## SUMMARY

Investigations on the fecal transmission of *T. spiralis* have been carried out using fox cubs and albino rats as donors, while swine, albino rats and fox cubs served as recipient hosts. The feces of experimentally infected animals have been examined by the Baermann technique to ascertain the number of larvae eliminated.

The following factors were found to affect transmission: (1) time interval between infection and passage of feces by donor, (2) relative immunity of donor, and (3) number of trichinae ingested by the donor. About 95% of the larvae passing through the intestinal tract of the donors were recovered from feces eliminated within 24 hours after ingestion. Immune animals eliminated a larger percentage of the administered trichinae than did nonimmune animals.

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# *TRICHOSTRONGYLUS DREPANOFORMIS* N. SP. (NEMATODA: TRICHOSTRONGYLIDAE) FROM THE DUODENUM OF THE SHEEP (*OVIS ARIES*)

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The following description of a new species of the genus *Trichostrongylus* is based on 2 males which were recovered from the duodenum of a sheep. This animal had been grazing for some months at the McMaster Field Station, Badgery's Creek, New South Wales, but prior to this it was located near Cunnamulla in south-west Queensland, Australia.

*Trichostrongylus drepanoformis* n.sp.

*Description:* Males about 4.6 mm long and taper gradually from posterior end; width immediately anterior to caudal bursa, 0.1 mm. Head 0.01 mm wide with 3 inconspicuous lips; buccal cavity minute; simple esophagus about 0.72 mm long. Excretory pore lies in a depression in cuticle about 0.16 mm from anterior end and immediately behind circumenteric nerve ring. Cuticle marked by very fine transverse striations between 2 and 3 microns apart, minute pre-bursal papillae present.

Bursa with 2 prominent lateral lobes, distal ends curled inwards. Ventro-ventral ray thinner and shorter than latero-ventral ray and diverges from it. Latero-ventral ray lies close to lateral rays and about same thickness as antero-lateral ray, but remaining lateral rays alike and thinner than antero-lateral ray; tip of antero-lateral ray relatively blunt, whereas all others except dorsal ray taper gradually to a small rounded end. Externo-dorsal rays curve outwards from base of dorsal ray and approximately equal to it in length.

Dorsal ray 35 microns long and bifurcates at a point about ⅔ of its length from base; each bifurcation in turn sub-divided into a short outer arm and a long inner arm; main stem of ray measures 25 microns from base to point of bifurcation, where it is 7 microns wide.

Each spicule bent near middle to form an angle of approximately 90°. Anterior portion of each spicule 80 microns long and lies in longitudinal axis of worm; posterior portion 60 microns long, directed ventrally, thin, tapered and near tip curved posteriorly. Proximal ends of spicules each have a disc-like process, plane of which, in left spicule, directed transversely and in right spicule, antero-posteriorly. Semi-transparent alae project from dorsal surface of anterior half of each spicule.

Gubernaculum approximately 77 microns long. In lateral view entire dorsal edge smooth, but ventral edge irregular. In dorsal view gubernaculum thicker at posterior end, and more opaque at margins than at center.

Female and larval stages are unknown.

*Host: Ovis aries.*

*Habitat:* Duodenum.

*Locality:* Eastern Australia, either south-west Queensland or near Sydney.

*Holotype and Paratype:* Australian Museum, Sydney, W3710 and W3711. Both specimens damaged during examination.

The specimens on which this description is based were associated with *T. colubriformis, T. vitrinus, T. rugatus* and *T. probolurus.* The females of these and other species of *Trichostrongylus* are not readily differentiated from one another and it was not surprising that a search failed to detect any females which might belong to the new species.

The genus *Trichostrongylus* has been examined by Skrjabin et al (1954). These authors list 15 species which have been found in sheep but none of these possess spicules resembling those of *T. drepanoformis.* Three species, *T. thomasi, T. tenius*

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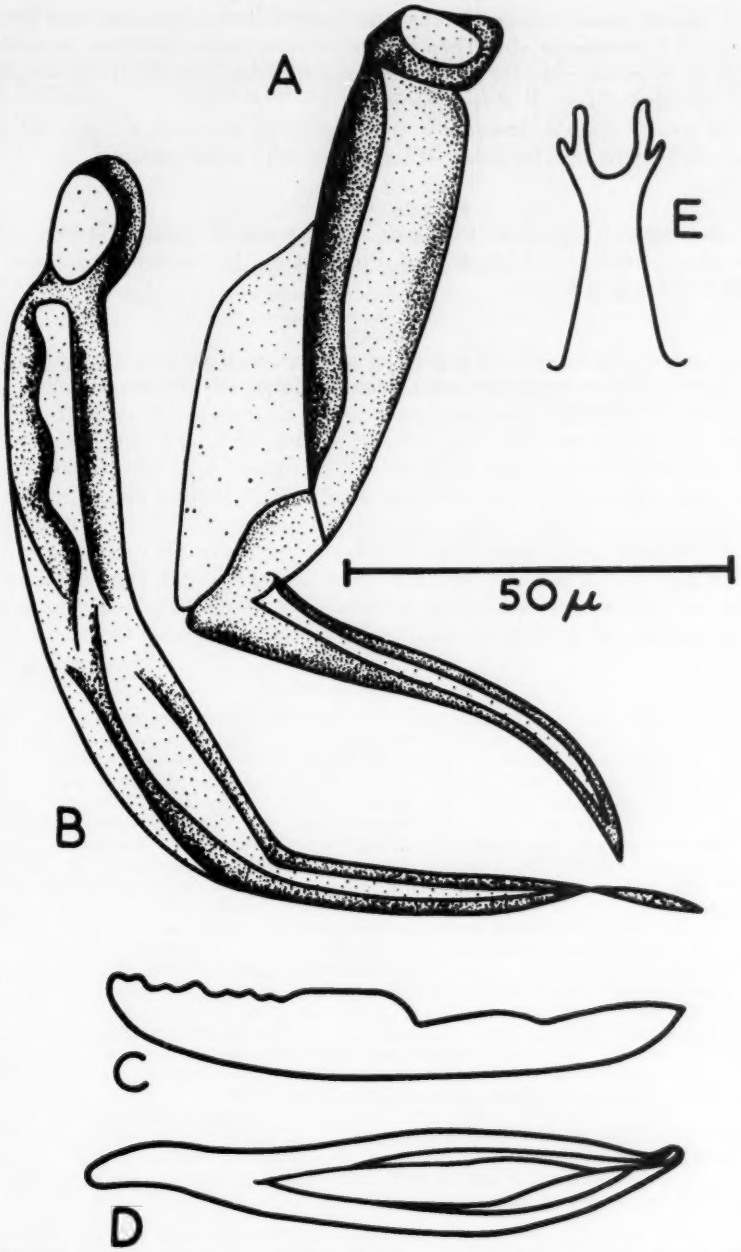


FIGURE 1. *Trichostrongylus drepanoformis* n. sp. A. Left spicule in lateral view. B. Right spicule. This has been twisted so that it is seen partly from the side and in part, dorsally. In lateral view it closely resembles the left spicule. C. Gubernaculum, lateral view. D. Gubernaculum, dorsal view. E. Dorsal ray.

and *T. medius,* possess spicules in which the posterior half is bent somewhat like the spicules of *T. drepanoformis.* These species, however, have never been recorded in sheep. Furthermore, in all 3 the spicules, gubernaculum and dorsal ray are otherwise quite distinct from *T. drepanoformis.*

The specific name *drepanoformis,* from the Greek drepanon, a sickle, has been chosen with reference to the sickle- or scythe-like shape of the spicules.

ACKNOWLEDGMENTS

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# PARASITOLOGIC SURVEYS IN CALI, DEPARTAMENTO DEL VALLE, COLOMBIA. IV. EXPERIMENTAL STUDY IN GUINEA PIGS OF THE PATHOGENIC CAPACITY OF *ENTAMOEBA HISTOLYTICA* FROM INFECTED PERSONS IN WARD SILOÉ, CALI*

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In connection with a parasitologic survey of families in ward Siloé, Cali, Colombia, special interest has developed in the incidence and significance of *Entamoeba histolytica* among the individuals whose feces have been examined. It has been demonstrated that about 40% of these persons harbor this ameba, that 4/5 of the strains belong to the large race and 1/5 to the small race, and that these two races are distinguishable not only by significant mean size but also by morphologic characteristics (Faust, 1958). Moreover, insanitary environmental conditions and almost complete lack of personal hygiene provide opportunity for constant reinfection. Nevertheless, with rare exceptions these infections are clinically non-pathogenic (Faust and Giraldo, 1958). It has, therefore, seemed important to test these amebas in a susceptible experimental host to determine whether or not they are potentially pathogenic.

## MATERIALS AND METHODS

A total of 14 strains of *Entamoeba histolytica* was tested. The amebas were recovered as cysts from formed or semiformed stools of the persons under investigation. Their average sizes and morphologic characteristics were determined, as follows: Measurement of cyst diameters was made both on direct fecal films and after the technic of zinc sulfate centrifugal flotation (Faust, 1958) was employed. When many cysts were available, both size range and mean diameter were obtained; if only 1 cyst or a very few cysts of the same size were present, a single measurement was recorded. (See Table I.) All large-race cysts on unstained films contained 1 to 4 large sausage-shaped chromatoidals with rounded ends; the small race cysts usually contained a cluster of small grape-shaped chromatoidals (strains 1563, 1568, 1641), rarely a single short sausage-shaped chromatoidal (strain 1564).

The cysts were concentrated in an International clinical centrifuge at 1500 rpm after the feces had been comminuted 1:10 in water and screened through 2 layers of moist surgical gauze. Repeated centrifugal washings removed the coarse débris and a majority of the associated bacteria. The cysts, relatively free of bacteria, were used directly for the feeding experiments; for intracecal inoculation with trophozoites the cysts were planted in Balamuth's monophasic culture medium and the cultures incubated at 37°C. Transfers were made every 48 hours. One thousand units each of penicillin and streptomycin were added to each culture tube to control overgrowth of bacteria. When luxuriant growth of the trophozoites had been obtained following 2 or more subcultures, the amebas were concentrated in physiologic salt solution, their numbers calculated, and they were used for inoculation.

Cysts of 6 strains were fed to 38 guinea pigs, in the amount of 10,000 to 200,000 per animal (Table I). Two of the strains contained only the large race, 3 contained both large and small races, and 1 only the small race of *E. histolytica*. Trophozoites of 10 strains were employed for intracecal inoculation into a total of 115 animals in 16 test series, in the amount of 40,000 to

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200,000 trophozoites per animal (Table I). Nine of these strains consisted only of the large race and 1 contained both large and small races. Strains 1641 and 1642 had previously been utilized as cysts in feeding tests. The intracecal inoculum was in each case concentrated into 1 ml.

With very few exceptions all of the guinea pigs utilized were young (150 to 200 gm weight). Throughout the experiments they were fed only carrots and cabbage. Food was withheld for 24 hours preceding oral or intracecal inoculation. Four to 15 animals were employed in each series. The technic of intracecal inoculation was essentially the same as that described by Carrera and Faust (1949).

The 38 guinea pigs utilized for the feeding tests survived the period of 7 to 10 days at which time they were sacrificed under tribromoethanol (Avertin) anesthesia, and are all counted in the autopsy examinations. Of the 115 animals employed in the intracecal inoculations, 12 died of faulty operative technic or succumbed during the night and were not satisfactory for autopsy examination, hence the net number in this group is 103. The interval between intracecal inoculation and sacrifice varied from 6 to 11 days, but several guinea pigs which had just died at earlier intervals and were in good condition for examination are included. The living animals in the intracecal series were sacrificed under tribromoethanol anesthesia. Autopsy examination consisted in opening of the peritoneal cavity, then macroscopic examination of the abdominal viscera, followed by removal of the entire intestinal tract and liver. The intestines were then opened lengthwise and studied for evidence of ulceration, particularly at the level of the distalmost portion of the ileum, the cecum and adjacent portion of the colon. Microscopic examination was made of fecal material from the lumen and of superficial scrapings from suspected lesions in the mucosa of these segments of the intestine. Blocks of tissue from these levels and from liver were fixed in Zenker-formol fluid for subsequent sectioning, staining and examination.

## RESULTS

In addition to summary tabulation of the data obtained (Table 1) the comments that follow are considered to be essential for an evaluation of the autopsy findings.

### *Oral Inoculation with Cysts*

The number of tests in these series (6 strains fed to 38 animals) was probably inadequate to determine the tissue-invading potential of these strains. Moreover it is possible that an incubation period of 7 to 10 days is not sufficient to allow excystation, metacyclic division, growth and multiplication, so as to provide a satisfactory evaluation of the ability of *E. histolytica* to colonize the cecum of the guinea pig following the feeding of cysts, and not long enough to determine the tissue-invasive capacity of these strains. Nevertheless, in 1 or 2 animals of each series active trophozoites were found in fresh fecal films from the cecal feces and from superficial mucosal scrapings, and in 1 animal (strain 1563), and possibly in another (strain 1642), trophozoites had invaded the mucosa. As the experiment was set up there was no way of knowing what percentage of inoculated cysts proceeded to excyst or the exact location of excystment, nor was there a direct test of the relative viability of the cysts of the respective strains which were utilized after they had passed through the stomach. Yet there was recovery of active trophozoites in each series except 1568, irrespective of the number of cysts fed, suggesting that in the young guinea pig 10,000 viable cysts may be sufficient to determine the capacity of the ameba to become established at the cecal level. There is also an indication that macroscopic evidence of lesions is a poor criterion of initial amebic invasion; likewise 1 series of serial sections may fail to reveal actual incipent penetration at other sites where macroscopic inspection is negative.

### *Intracecal Inoculations*

Of the 115 animals utilized in these tests, 103 were satisfactory for autopsy examination. In 64 there was direct proof that the amebas had colonized in the

cecum, and in at least 53 that they had invaded the mucosa. In several instances extensive cecal ulceration was covered with a pseudodiphtheritic membrane, a not unusual accompaniment of fulminating cecal amebiasis in the guinea pig (Carrera and Faust, 1949). It seems probable that the calculated incidence of 51.4 to 54.3% tissue invasion by these 10 strains is conservative, and that more sections of involved or suspicious areas would have increased the percentage. Moreover for each strain tested 1 or more animals demonstrated tissue-invasive capacity of the ameba, although different series of the same strain showed considerable variation and different strains appeared to manifest some difference in invasive capacity (cf. strain 1711 vs. strain 1774). Within the limits of the numbers of trophozoites inoculated (40,000 to 200,000) there appeared to be no significant variation in the percentages of colonization and tissue invasion.

TABLE I. *Summary of guinea pig inoculation tests with strains of* Entamoeba histolytica *of human origin from Siloé, Cali, Colombia.*

| Strain no. | | Size of cysts (microns) | No. E.h. inoculated (each animal) | Interval between inoculation and autopsy (in days) | No. animals examined at autopsy | No. positive | No. with tissue invasion |
|---|---|---|---|---|---|---|---|
| A. CYSTS (per os) | | | | | | | |
| 1563 L + S | | 12.4–14.5 (L) 8.3– 8.8 (S) | 15,000 | 8(7) | 7 | 2 | 1 |
| 1564 S | | 7.2– 8.8 | 50,000 | 10(7) | 7 | 1 | 0 |
| 1568 L + S | | 10.35 (L) 7.8 (S) | 40,000 | 7(7) | 7 | 0 | 0 |
| 1569 L | | 9.8 | 10,000 | 10(4) | 4 | 1 | 0 |
| 1641 L + S | | 9.8–14.5 (L) 7.8 (S) | 200,000 | 9(7) | 7 | 2 | 0 |
| 1642 L | | 9.8–13.5 | 200,000 | 9(6) | 6 | 1 | 1 (?) |
| | | | | | 38 | 7 (19.4%) | 1 (+1?) (2.6 or 5.2%) |
| B. TROPHOZOITES (Ic) | | | | | | | |
| 1522 L | (Ser. 1) | 11.4–14.5 | 50,000 | 8(4) | 4 | 2 | 2 |
| | (Ser. 2) | | 190,000 | 2(3), 7(1) | 4 | 4 | 4 |
| | (Ser. 3) | | 100,000 | 3(5) | 5 | 3 | 2 (or 3?) |
| 1523 L | (Ser. 1) | 12.4–14.5 | 40,000 | 2(2), 3(1), 8(12) | 15 | 7 | 7 |
| | (Ser. 2) | | 100,000 | 2(1), 8(3) | 4 | 4 | 3 (or(4?) |
| | (Ser. 3) | | 200,000 | 3(2), 9(1) | 3 | 3 | 3 |
| 1641 L + S | | (see above) | 160,000 | 4(1), 9(8) | 9 | 5 | 4 |
| 1642 L | | (see above) | 200,000 | 10(4), 11(6) | 10 | 6 | 6 |
| 1647 L | | 11.4–13.5 | 120,000 | 9(2), 10(7) | 9 | 4 | 4 |
| 1670 L | (Ser. 1) | 12.4–14.5 | 60,000 | 3(1), 8(1), 9(2) | 4 | 3 | 1 |
| | (Ser. 2) | | 100,000 | 4(1), 7(1), 11(2) | 4 | 2 | 0 |
| 1672 L | (Ser. 1) | 10.4–14.5 | 120,000 | 9(4) | 4 | 4 | 4 |
| | (Ser. 2) | | 100,000 | 4(1), 11(4) | 5 | 2 | 2 |
| 1711 L | | 12.4–15.5 | 100,000 | 2(1), 3(1), 6(1), 8(1), 11(4) | 8 | 7 | 5 |
| 1771 L | | 13.5–14.5 | 100,000 | 3(2), 4(1), 7(2), 11(2) | 7 | 5 | 5 |
| 1774 L | | 9.5–15.2 | 100,000 | 6(4), 11(4) | 8 | 3 | 1 (or 2?) |
| | | | | | 103 | 64 (62.1%) | 53 (51.4%) to 56 (54.3%) |

Numbers in parentheses, 4th column = animals autopsied on that day.
L, large race; S, small race; Ic, intracecally.

## DISCUSSION

Since clinical and laboratory evidence accumulated over a period of 2 years has consistently indicated that infections with *Entamoeba histolytica* in the human population under investigation are essentially asymptomatic, even though the strains are predominantly of the large race, it became an important corollary to discover if these strains were also non-pathogenic for the guinea pig, a sensitive test animal for this organism. Neal (1957) has commented that "guinea pigs react in the same

way as rats to infections with virulent and avirulent strains. They differ from rats in that the infection with virulent strains is lethal."

Our series of tests with guinea pigs has demonstrated that, between the 2nd and 11th day after intracecal inoculation with trophozoites, 64 of 103 (62.1%) of the animals necropsied had active cecal infection, and of the infected ones at least 83.0% showed invasion of the cecal wall. Seven (19.4%) of the 38 guinea pigs necropsied 7 to 10 days following the feeding of cysts provided evidence of colonization, but only 1, or possibly 2, demonstrated tissue invasion. This moderate degree of colonization and low percentage of tissue invasion following the ingestion of cysts are probably due to a small number of viable cysts which survived passage through the gastric juice of the stomach compared with the number of cysts fed, hence the meager number which had an opportunity to reach the terminal ileum and cecum and to excyst (Tsuchiya, 1939). Even on a fasting stomach, guinea pigs from the same breeding stock as those which were utilized by us had a pH of 2 to 2.4. Even though food was withheld from our guinea pigs for 24 hours before cysts were introduced, the intestinal tract, including the cecum, contained an appreciable amount of partly digested food. Hence, it is quite likely that relatively few metacystic trophozoites produced by excystation of the surviving cysts in the terminal ileum and cecum had a chance to apply themselves to the surface of the cecal mucosa or to become lodged in the cecal crypts, locations which apparently need to be reached in order for *E. histolytica* to colonize.

The much higher percentage of colonization resulting from introduction of thousands of active trophozoites directly into the cecum might have been anticipated, since some of these many vigorous trophozoites undoubtedly had an early opportunity to secure lodgement on the cecal mucosa, to multiply, and in some instances to initiate mucosal penetration as early as 48 hours after inoculation. Yet considering the luxuriant growth of several species of protozoa (flagellates, *Entamoeba cobayae, Balantidium caviae*) and bacteria which were natural lumen inhabitants of the cecum of the guinea pigs utilized, it is perhaps remarkable that an exotic competitor like *E. histolytica* was able to establish itself in the circumscribed environment. The explanation may lie in the fact that *E. histolytica* in the guinea pig is essentially a tissue invader, whereas the native protozoa are lumen-dwelling commensals feeding exclusively on the cecal contents.

While colonization of *E. histolytica* in the guinea pig's cecum necessarily precedes tissue invasion, the primary objective of our study was to determine the tissue-invasive potential of the strains under experimental investigation. In some of the guinea pigs submitted to intracecal inoculation the amebic lesions consisted of small discrete cecal ulcers, confined to the outer portion of the mucosa; in other guinea pigs there were solitary or multiple centers of invasion of the cecum involving the base of the mucosa, and in 1 animal (strain 1711) the amebas had advanced into the muscle layers. In the more fulminating infections the entire cecum, once the adjacent portion of the colon (strain 1647) and twice the terminal segment of the ileum (strains 1647, 1711) partook of extensive amebic ulceration. Only once in sections of the cecum was there evidence of bacterial involvement at the depths to which the amebas had penetrated (strain 1523, 1st series). Some animals in the intracecal inoculation tests had macroscopically intact mucosae, yet on section demonstrated numerous incipient amebic ulcers, and in 1 guinea pig there was deep cecal ulceration without superficial evidence of amebic infection. In this respect

cecal amebiasis in the guinea pig parallels that in man. In 1 animal the trophozoites recovered from mucosal lesions belonged to the small race (strain 1641).

A condition comparable to that which has been found in the present study of human amebiasis in a ward of Cali, Colombia, existed in a children's home in New Orleans, Louisiana, two decades ago, where *E. histolytica* infection was highly endemic (Ivanhoe, 1943), but without clinical evidence of the infection. Sixteen different strains of this ameba were inoculated intragastrically as cysts or intracecally as trophozoites into 26 grown dogs, all of which became infected. Four developed acute dysentery with extensive amebic invasion; 6 had temporary dysentery with amebic ulceration; 3 developed amebic ulcers without dysentery, and 13 had typical amebic ulcers, although the amebas were not demonstrable (Tobie, 1940).

In so far as we have been able to discover, no study has been made of the process of excystation of *E. histolytica* following the feeding of cysts to guinea pigs. Hegner, Johnson and Stabler (1932) provided a few observations on 2 Panamanian monkeys to which they had fed cysts. Excystation occurred within 2⅓ hours in the small intestine of 1 animal and within 3 hours in the other animal. Swartzwelder (1939) concluded on the basis of his observations that, in the dog, excystation of *E. histolytica* of human origin occurred in the ileum and large intestine in not less than 4 hours. Moreover, apparently viable cysts were recovered in the intestinal contents of dogs sacrificed 13 to 16 hours after inoculation with cysts. At 24 hours trophozoites were first recovered from mucosal scrapings taken from the surface of minute cecal ulcers. Tsuchiya (1939) reported that, in rats with empty stomachs, within 1 hour after inoculation cysts were recovered all the way from the stomach to the cecum, at 2 hours from the middle ileum to the cecum, while at 4 to 8 hours they were excysting in the distalmost part of the ileum and cecum. Beaver et al (1956) working with dogs, guinea pigs and rats, concluded that the carrier strain, with which they had produced asymptomatic infection in all of 42 human volunteers, "could not be regarded as non-pathogenic, since it produced typical amebic lesions in dogs, guinea pigs and rats, and was as pathogenic in guinea pigs and rats as a recently isolated strain from a frankly dysenteric patient." Okamoto (1953) fed cysts from 20 indigenous Japanese strains and 10 introduced tropical strains of *E. histolytica* (all large race) to laboratory rats, obtaining 34.5% colonization with the native strains and 53.1% with the tropical strains. Stam (1958) compared his findings for *Entamoeba invadens* in reptiles with Okamoto's for *E. histolytica* in rats, and suggested that "larger cysts may be expected to produce more virulent amoebae than smaller cysts," . . . while "the smallest cysts produce the amoeba most effectively adapted to poor environmental conditions."

With the few exceptions noted, the strains which we have tested all belong to the large race (Table 1), with cyst diameters varying from 9.5 to 15.5 microns, whereas strains of the small race tested had a size range between 7.2 and 8.8 microns. In the human population in our survey group strains of both large and small races produce essentially asymptomatic infections, as in the infection of human volunteers by Beaver et al. (1956), yet at least 51.4% of our inoculated guinea pigs demonstrated amebic invasion of the cecum varying in degree from moderate to fulminating invasion. All strains of the large race, irrespective of their size ranges, produced amebic lesions of the cecum.

Our series of tests in guinea pigs provides suggestive evidence in line with the

results obtained by Frye and Meleney (1933), Phillips and Bartgis (1954) and Neal (1957), that "*E. histolytica* trophozoites are per se tissue invaders and are not dependent on previous erosive damage to the mucosa produced by inert objects or by other microorganisms."

Finally, our study demonstrates that residence of *E. histolytica* in the large intestine of an indigenous human population in a tropical area in Colombia without clinical manifestations of amebiasis has not incapacitated this ameba to invade the cecal wall of the guinea pig and produce moderate to fatal ulceration.

## SUMMARY

1. To test the pathogenic potential of 14 strains of *Entamoeba histolytica,* obtained from essentially asymptomatic persons living in an unsanitated ward of Cali, Colombia, young guinea pigs were inoculated per os with cysts from 6 strains and intracecally with trophozoites from 10 strains.

2. Concentrates of washed cysts from 6 strains, in the amount of 10,000 to 200,000 were fed to each of 38 guinea pigs in groups of 4 to 7 per series. Seven to 10 days later the animals were sacrificed. Colonization in the cecum had occurred in 7 (19.4%), and in 1, and possibly a 2nd animal, mucosal invasion of the cecal wall had been initiated.

3. Trophozoites derived from cysts of 10 strains which had been cultured in Balamuth's medium were inoculated intracecally in the amount of 40,000 to 200,000, into each of 115 guinea pigs in 16 series, 4 to 15 animals per series. At necropsy of 103 of these animals 2 to 11 days later, direct examination and available sections revealed colonization in 64 (62.1%) and tissue invasion in 53 (51.4%). The latter varied from initial mucosal erosion, with solitary or multiple amebic ulcers, to deep penetration usually confined to the cecum, once involving the adjacent colon and twice the terminal ileum. Pseudodiphtheritic membranes had developed over the fulminating lesions.

4. Although these strains of *E. histolytica* (all but 1 consisting exclusively or primarily of the large race) were obtained from asymptomatic carriers, the present study has demonstrated their relatively high pathogenic potential in the test animals.

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(continued from p. 440)

TABLE I. *Comparison of larval trematode infections in* Nassarius obsoletus (*Say*) *collected from low-tide and high-tide zones.*

| Species of trematode | Low-tide zone | | | | | | | | | | High-tide zone | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10-6-51 | 11-6-51 | 12-2-51 | 1-12-52 | 2-8-52 | 3-8-52 | 3-21-52 | 4-14-52 | 5-14-52 | 6-22-52 | 3-21-52 | 4-14-52 | 5-14-52 | 6-22-52 |
| *Zoogonus lasius* | 12 | 30 | 28 | 11 | 14 | 24 | 116 | 19 | 40 | 3 | 213 | 180 | 69 | 21 |
| *Himasthla quissetensis* | 4 | 15 | 6 | 5 | 3 | 8 | 43 | 12 | 18 | 5 | 147 | 105 | 21 | 33 |
| *Austrobilharzia variglandis* | 2 | 1 | 2 | 0 | 1 | 1 | 20 | 6 | 4 | 0 | 55 | 29 | 7 | 0 |
| All other trematode larvae | 0 | 5 | 6 | 4 | 2 | 6 | 4 | 2 | 6 | 0 | 12 | 17 | 12 | 6 |
| Double infections | 0 | 3 | 0 | 0 | 0 | 1 | 4 | 0 | 0 | 0 | 15 | 27 | 2 | 0 |
| Total infected | 18 | 54 | 42 | 20 | 20 | 40 | 187 | 39 | 68 | 8 | 442 | 358 | 111 | 60 |
| Total examined | 495 | 488 | 490 | 324 | 573 | 521 | 436 | 497 | 251 | 224 | 586 | 438 | 250 | 144 |
| Percent infected | 3.6 | 11.0 | 8.6 | 6.2 | 3.5 | 7.7 | 43.0 | 7.9 | 27.1 | 3.6 | 75.4 | 81.8 | 44.2 | 41.5 |

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# MORPHOLOGY AND VIRULENCE OF *TRYPANOSOMA CRUZI*–LIKE HEMOFLAGELLATES ISOLATED FROM WILD MAMMALS IN GEORGIA AND FLORIDA

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During epizootiological investigations of leptospirosis in wild animals in southwestern Georgia and northwestern Florida, hemoflagellates resembling *Trypanosoma cruzi* were recovered from 103 of 1584 animals collected (Brooke et al, 1957; McKeever et al, 1958). The organisms were found in cultures of kidney tissue from 17 percent of 552 opossums, 1.7 percent of 118 grey foxes, 1.5 percent of 608 raccoons and 1.1 percent of 306 striped skunks. Triatomid bugs were found only rarely in the areas where the animals were collected, and no human cases of Chagas' disease have been reported to date.

*T. cruzi* and *T. cruzi*-like organisms have, however, been isolated from several species of *Triatoma* in the United States (Wood and Wood, 1941; Sullivan et al, 1949; and Usinger, 1944). Species of wood rat, wood mouse, deer mouse, opossum, bat, armadillo (Usinger, 1944; Wood, 1952) and raccoon (Walton et al, 1958) have been reported as reservoir hosts. Identifications of the organisms were usually based on the morphology and cyclical development in insect and mammalian hosts. Studies of virulence and infectivity rates of these organisms have been hampered by the great variations in susceptibility of laboratory animals toward infection with *T. cruzi* (Mazzotti, 1940; Hauschka, 1949; Pizzi et al, 1949). Although there is some experimental evidence that wild animal hemoflagellate isolates can infect domestic animals, man and other primates (Diamond and Rubin, 1958; Packchanian, 1943; Davis, 1943), it is still not known whether any or all are capable of producing Chagas' disease. Nor is it known whether the virulence of animal strains can be increased by passage through new hosts under natural conditions. Serological tests for the identification of species and/or strains of hemoflagellates have had limited use (Hauschka, et al, 1950; Packchanian and Turck, 1949; Senekjie, 1943; Walton, et al, 1958).

The present report concerns the comparative study of 7 strains of hemoflagellates isolated during a leptospirosis field study. In order to identify the wild animal isolates, their characteristics were compared with those of 3 established species of *Trypanosoma;* namely, *T. cruzi, T. rangeli,* and *T. duttoni.*

## MATERIALS AND METHODS

Seven strains of animal hemoflagellates were selected for investigation, 5 from opossum (FH4, FH5, FH6, OR21 and FR4) and 1 each from raccoon and skunk. For comparison, 2 human strains of *T. cruzi* (Corpus Christi and Brasil), *T. rangeli* isolated from *Triatoma protracta* in Venezuela, kindly supplied by Dr. R. G. Yaeger of Tulane University, and *T. duttoni,* a non-lethal mouse hemoflagellate, were studied. The organisms were maintained in culture, and the morphology of the various stages noted and compared. Inoculation of the cultured organisms into young mice permitted comparisons of infectivity rates as well as examination of

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the morphology of mammalian stages of the parasites. To determine whether vectors of Chagas' disease could be infected and transmit the organisms, triatomid species were permitted to feed on infected mice, and the intestinal contents of infected bugs inoculated into mice. Finally, blood from the infected mice was inoculated into a culture medium. Standard methods were followed and all strains were treated in the same manner, except as noted.

*In vitro cultivation:* The animal isolates were transferred from leptospira medium to a diphasic blood medium with overlay of buffered saline (Offutt, 1946) used routinely for hemoflagellate cultures. Control cultures of *T. cruzi, T. rangeli* and *T. duttoni* had been maintained on this medium for from several months to several years in our laboratories. All cultures were incubated at 24–25° C and transferred every 14 days to freshly prepared blood medium. Wet mounts of the cultures were examined after 4, 7, 10, 14 and 28 days incubation. The stages present, motility, relative numbers and other cultural characteristics were noted. Organisms immobilized and supravitally stained with Beemer's solution were measured under oil immersion magnification with an ocular micrometer. Lengths of undulating membranes and free flagella were also recorded for comparisons. Measurements of at least 100 crithidial forms in 10-day-old cultures of each strain were used to calculate mean lengths. A year later, 25 additional crithidia were measured to determine whether any changes had occurred in the mean lengths during in vitro cultivation. Wet thin smears of culture overlay were fixed in osmium fumes, air dried and stained with dilute Giemsa at pH 6.0 to 6.4. Lengths of stained organisms were measured and location of nuclei and kinetoplasts determined.

The formula of the modified Beemer's solution employed was as follows: Sodium citrate, 1.1 g; sodium chloride, 0.65 g; mercuric chloride (sat. sol.), 0.10 ml; brilliant cresyl blue, 0.05 g; methylene blue, 0.04 g; distilled water, 100 ml. One drop was allowed to run under cover slip of a wet mount of cultured organisms.

In reestablishing strains in culture from mice which had become infected with forms from culture or insects, blood was removed aseptically from the heart and inoculated in 0.1 to 0.2 ml amounts into tubes of the medium. Cultures were examined at weekly intervals for plaques of non-flagellated organisms or motile stages. Tubes were held 28 days and considered negative if no hemoflagellates were observed.

*Animal inoculation:* Inocula were prepared by pooling overlay from several 10-day-old and 28-day-old cultures. Suspensions estimated as "heavy" contained many metacyclic trypanosomes as well as crithidia. Animals were inoculated via the intramuscular technique described by Wood (1949) or by intraperitoneal injection. With Wood's method, 0.1 ml of inoculum was injected into the upper leg or lower back muscles of 3-week-old male CFW (Carworth Farms Webster) mice. Animals were sacrificed after 8 and 14 days, and impression smears made from tissue excised from the site of inoculation. Smears were air dried and stained with Giemsa.

Intraperitoneal inoculations were made into male mice of CFW and C3H strains. The CFW mice were known to be relatively resistant to infection with *T. cruzi* and *T. rangeli*, but susceptible to *T. duttoni*. The C3H mice were considered to be susceptible to *T. cruzi* by Pizzi and Prager (1953). The mice varied in age from 2 weeks to adulthood. In each experiment, groups of 4 to 10 mice of the same age and sex were inoculated with 0.1 ml of a suspension of each hemoflagellate strain except *T. duttoni*. Previous experience had shown that all mice inoculated with *T. duttoni* acquired transient infections with many organisms in the blood between the 2d and 3d weeks. In these experiments, only 6 animals were inoculated with *T. duttoni* to supply material for morphological comparisons of the mammalian stages of the hemoflagellates. Half of the mice in each group were inoculated subcutaneously with variable doses (1 to 2 mg) of cortisone acetate either before or 1 to 4 times after injection with the protozoa (Wolf et al, 1953). Groups of mice inoculated with cortisone and culture medium served as controls. Death and infectivity ratios of both cortisone-treated and untreated animals were compared.

Presence of infection was determined by examining microscopically drops of blood for the presence of trypanosomes at 4 day intervals following inoculation. Each mount was examined for 3 minutes and the numbers of organisms counted or estimated. Wet blood smears were fixed in osmium fumes and stained with acid Giemsa. A few mice were killed at various times following inoculation, and heart, spleen and kidneys removed and fixed for sectioning. Tissue sections stained with Harris' hematoxylin were examined for leishmanial stages of the hemoflagellates.

*Insect studies:* Laboratory raised *Triatoma infestans* and *Triatoma uleri* were permitted to feed on mechanically immobilized infected mice. Dejecta expelled following blood meals 1 month and 2 months later were examined for the presence of crithidia and metacyclic trypanosomes. Wet fecal smears were fixed with osmium fumes and stained with Giemsa. Infectivity of the organisms was determined by intraperitoneal inoculation of fecal suspensions or macerated insect abdomens into 3-week-old mice treated with cortisone.

## RESULTS

*Cultivation.* Both crithidia and metacyclic trypanosomes were present in the original cultures of wild animal kidney tissue in leptospira medium. The cultures were over 28 days old when received, and the organisms were pleomorphic, vacuolated and often moved sluggishly. The organisms from opossum, skunk and raccoon appeared identical.

In the first cultures after transfer to hemoflagellate medium, and in cultures from infected mouse blood, typical *cruzi*-type clumps or "plaques" of non-flagellated organisms (diameter 6–10 microns) appeared between the 4th and 10th day. Numbers and size of the plaques seemed to be more dependent upon the number of organisms in the initial inoculum than on the source of the strain. By the 14th day, cultures usually contained a mixture consisting of a few plaques, moderate numbers of short, sturdy crithidia and, many slender crithidia. A few metacyclic trypanosomes were occasionally present, but these forms were not found regularly until after several transfers or in the 28-day-old cultures. All 3 stages were present in cultures of the isolates, *T. cruzi* and *T. rangeli*; only 2 were seen in *T. duttoni* cultures (table I).

TABLE I. *Hemoflagellates in culture on diphasic blood medium.*

| Organisms | Non-flagellated stage present or absent | Crithidial stage | | | Trypanosomal stage Range of length microns |
|---|---|---|---|---|---|
| | | 100 organisms | | 25 organisms* | |
| | | Range of length microns | Mean length microns | Mean length microns | |
| *Isolates from:* | | | | | |
| Opossum FR4 | present | 20.0 to 44.4 | 25.6 | 25.6 ± 1.4 | 16.7 to 27.8 |
| Opossum FH4 | present | 16.7 to 38.9 | 24.0 | 24.4 ± 1.2 | 15.5 to 24.4 |
| Opossum FH5 | present | 20.0 to 33.3 | 24.5 | 25.0 ± 1.0 | 13.3 to 22.2 |
| Opossum FH6 | present | 22.2 to 44.4 | 26.6 | 26.0 ± 1.2 | 13.3 to 22.2 |
| Opossum OR21 | present | 16.7 to 33.3 | 22.8 | 21.9 ± 0.7 | 20.0 to 22.2 |
| Raccoon | present | 16.7 to 38.9 | 26.0 | 24.3 ± 1.1 | 16.7 to 22.2 |
| Skunk | present | 20.0 to 44.0 | 26.7 | 27.3 ± 1.3 | 16.7 to 22.2 |
| *Controls:* | | | | | |
| *T. cruzi* (Corpus Christi) | present | 20.2 to 44 | 24.5 | 23.5 ± 1.0 | 16.7 to 24.4 |
| *T. cruzi* Brasil | present | 13.4 to 33.3 | 23.3 | 23.6 ± 0.9 | 11.1 to 22.2 |
| *T. rangeli* | present | 20.0 to 44.4 | 30.9 | 30.3 ± 1.3 | 20.0 to 24.2 |
| *T. duttoni* | absent | 10.0 to 18 | 15.7 | 15.9 ± 0.9 | 10.2 to 16.6 |

* Measured 1 year later.

Measurements of the length of crithidia and metacyclic trypanosomes revealed no appreciable differences between the mean lengths and ranges recorded for the isolates and *T. cruzi* (table I). *T. rangeli* was longer and *T. duttoni* shorter in length. There was no significant difference between the means of 25 organisms measured over a year after the initial study.

While dividing organisms were common in the non-flagellated and crithidial stages, dividing trypanosomes were never seen. The relative number of organisms of each stage, size, rate of development and amount of pleomorphism varied in duplicate tubes of culture and from transfer to transfer of a single strain. In general, there was as much variation among the organisms of one strain as there was among the strains including the *T. cruzi* controls. *T. rangeli* and *T. duttoni* are readily identifiable in culture, and could be distinguished regularly from the *cruzi*-like hemoflagellates. Of all the isolates, the raccoon strain was the only strain to show repeated differences in culture. Up to the 7th day, crithidia of this strain appeared slightly longer, slenderer and more pointed (resembling *T. rangeli*

slightly). In the 10-day-old cultures, these differences had disappeared, and the cultures looked like the other *cruzi*-type organisms. There were more rosettes of crithidia in cultures of the raccoon strain than in any other culture except *T. duttoni.*

Examination of the osmium-fixed and stained cultured organisms showed the nuclei near the center. Kinetoplasts were usually large and varied in location from very near the anterior end in the short sturdy crithidia to near the posterior tip in the metacyclic trypanosomes. Variation in size and location of the kinetoplast is characteristic of rapid multiplication of organisms in culture. Relative measurements showed no differences in the strains of *cruzi*-type organisms. In contrast, kinetoplasts of *T. rangeli* and *T. duttoni* were consistently smaller, and the length of the free flagellum usually longer. There have been no recognizable morphological changes in the organisms of any of the 7 strains during 3 to 4 years of in vitro growth.

*Animal inoculations.* Stages and relative numbers of organisms in mouse tissue following intramuscular and intraperitoneal inoculations are shown in table II. The estimated numbers of parasites found are recorded from rare (±)

TABLE II. *Hemoflagellates in mouse tissue.*

| Organisms | Intramuscular inoculation | | Intraperitoneal Inoculation | | |
|---|---|---|---|---|---|
| | Organisms in impression smears | Trypanosomes in blood | Leishmania in organs | | |
| | | | Heart | Spleen | Kidney |
| *Isolates from:* | | | | | |
| Opossum FR4 | L ± | None | None | None | None |
| Opossum FH4 | L ± | ± | ± | ± | None |
| Opossum FH5 | L ± and T± | + | + | ± | None |
| Opossum FH6 | L ± | + | + | ± | None |
| Opossum OR21 | L ± and T± | + | + | None | None |
| Raccoon | L ± | ± | ± | None | None |
| Skunk | T ± | ± | ± | None | None |
| *Controls:* | | | | | |
| *T. cruzi* Corpus Christi | L + and T± | +++ | ++ | ± | None |
| *T. cruzi* Brasil | L ± | + | ± | + | None |
| *T. rangeli* | None | None | None | None | None |
| *T. duttoni* | None | ++++ | Not done | Not done | Not done |

L, leishmania. T, trypanosome. Number of parasites rare ± to abundant ++++.

to very abundant (++++). The *T. cruzi* (Corpus Christi) infections, which were the heaviest of the strains, produced few areas of leishmanial forms in the tissues and the animals receiving cortisone did not show increased invasion into heart or spleen tissues. A few leishmania were found in impression smears of teased muscle taken from the site of inoculation (8 days) with all but skunk, *rangeli* and *duttoni* strains. Trypanosomes were also present in the animals receiving the skunk, Corpus Christi and 2 opossum strains. The leishmania were morphologically indistinguishable for all the isolated strains and *T. cruzi.* Wood (1949) inoculated fecal material from infected triatomids intramuscularly and reported that the numbers of leishmanial and trypanosomal stages were more numerous in the tissues 14 days after inoculation than after 8 days. In our studies using the cultured organisms we noted, on the contrary, the organisms were present at the end of 8 days, but had practically disappeared from the tissues after 14 days.

Typical *T. cruzi* (Corpus Christi strain) infections could be produced fairly readily by intraperitoneal inoculation into C3H or 2-week-old CFW mice. The death rate was fairly high (15 of 24) (table III), and the course was characterized by rising parasitemia to death in 12 to 21 days. Mice surviving showed a peak

followed by a gradual decrease in the number of organisms in the blood. Cortisone increased the death ratio to 20 of 24 and increased the parasitemia markedly. *T. cruzi* (Brasil) and all the isolates except FR4 caused only transient infections with low parasitemias. Even in the few mice which died, the numbers of organisms in the blood was low. Parasitemias were slightly to moderately increased by inoculation of cortisone acetate. There was no peak, however, and the animals dying showed relatively few organisms in the blood. Table III shows infection and death ratios of all age mice inoculated both with and without cortisone. Opposum strain FR4 and *T. rangeli* did not produce detectable infections in any of the animals

TABLE III. *Infectivity rates in mice inoculated intraperitoneally with hemoflagellates.*

| Organisms | Mice without cortisone | | Mice with cortisone | |
|---|---|---|---|---|
| | Inf./inoc.* | Dead/inoc.** | Inf./inoc. | Dead/inoc. |
| *Isolates from:* | | | | |
| Opossum FH4 | 2/13 | 1/13 | 13/14 | 5/14 |
| Opossum FH5 | 6/13 | 0/13 | 13/14 | 4/14 |
| Opossum FH6 | 5/13 | 1/13 | 11/14 | 1/14 |
| Opossum FR4 | 0/11 | 1/11 | 0/14 | 4/14 |
| Opossum OR21 | 7/15 | 1/15 | 18/24 | 11/24 |
| Raccoon | 2/14 | 1/14 | 17/24 | 11/24 |
| Skunk | 2/12 | 1/12 | 15/22 | 9/22 |
| Control Mice | 0/10 | 1/10 | 0/18 | 5/18 |
| *Controls:* | | | | |
| *T. cruzi* Corpus Christi | 21/24 | 15/24 | 24/24 | 20/24 |
| *T. cruzi* Brasil | 4/13 | 0/13 | 11/16 | 3/16 |
| *T. rangeli* | 0/11 | 0/11 | 0/18 | 5/18 |

* Number infected/number inoculated. ** Number dead/number inoculated.

inoculated intraperitoneally. There was also no evidence of tissue invasion in heart, spleen, or kidneys of mice inoculated with these 2 strains. Organisms of all of the other isolates and *T. cruzi* invaded spleen and/or heart (table II). Kidney sections were all negative, and were examined only because the hemoflagellates had originally been isolated from minced kidney tissue.

Morphology of the trypanosomes in mouse blood showed similar variations in all the *cruzi*-type infections. Typically the predominating forms were fairly short and sturdy with a round central nucleus and a large posterior kinetoplast. There were also more slender forms with ovoid nuclei. Some of the latter had relatively small kinetoplasts. The slender forms were more abundant during the first few days of infection. Because the organisms were often scarce on a slide, sufficiently large numbers of trypanosomes of all the strains could not be measured. The impression was that there was as much variation between the organisms of a single strain as there was between strains including the control *T. cruzi* trypanosomes.

*Insect studies:* Nymphs of *T. infestans* fed on mice infected with strains FH4, FH5, FH6, skunk, raccoon and *T. cruzi* (Corpus Christi and Brasil) became infected as evidenced by the presence of crithidial and metacyclic trypanosomes in fecal droppings 30 and 60 days following feeding. Triatomids fed on mice inoculated with FR4 strain and *T. rangeli* did not develop infections. Dejecta from triatomids fed on OR21 strain mice contained only crithidia, and mice inoculated with this material and with macerated insect abdomens did not become infected. Since only a few insects were used with each strain, it is possible that trypanosomes of the OR21 strain were not found because the examinations were not made at the proper times. *T. uleri* were not infected with any of the strains. Passage through *Triatoma infestans* did not change the virulence of the strains for mice.

## DISCUSSION

The 7 isolated strains of hemoflagellates could not be distinguished morphologically in culture, in triatomids, or in mammalian tissues. There was also no evidence that opossum, skunk, raccoon and human strains of *T. cruzi* were morphologically different. There was a definite difference in the infectivity and virulence of various animal and control strains, making possible a division of the hemoflagellates into 3 groups on the basis of parasitemia and death rates of weanling CFW and C3H mice. Group I, a moderately virulent strain, includes only Corpus Christi. None of the animal isolates fell into this category. Group II, slightly virulent strains, includes Brasil strain (human) and opossum strains, FH5, FH6, and OR21. Brasil strain was originally virulent and has apparently lost some infectivity during the last 5 years in the laboratory. Group III, of very low virulence, includes skunk, raccoon and FR4 opossum strains.

Whether the wild animal strains present a potential public health hazard may depend on whether virulence can be enhanced fairly rapidly by animal passage. Gobel (1951) was able to restore Brasil strain to its original level of pathogenicity in about 2 years animal passage; the strain, however, was originally virulent for mice. The raccoon strains isolated in Maryland (Walton et al, 1958, and Diamond and Rubin, 1958) produced mild infections in a great variety of domestic and laboratory animals. Cardiac tissue from raccoon showed numerous lesions, but infections in domesticated animals were characterized by a lack of clinical symptoms and only 2 of 20 infected animals showed tissue stages.

Of particular concern to the diagnostic laboratory are the criteria by which hemoflagellates from non-human sources may be identified. Based on morphology and development in insects and mice, the seven strains studied can be assumed to be *T. cruzi.* Walton et al (1958) concluded from their morphological and serological studies that the organisms isolated from raccoons in Maryland are "conspecific with *Trypanosoma cruzi* (Chagas 1909) the causative agent of American human trypanosomiasis." Immunologic studies are being conducted with the present isolates and the human strains which may help determine the inter-relationship of these organisms.

## SUMMARY

Five strains of hemoflagellates isolated from opossums, 1 from a skunk and 1 from a raccoon were compared morphologically with each other and with known species of *Trypanosoma: T. cruzi, T. rangeli* and *T. duttoni.*

No differences in cyclic development and morphology in culture, mouse tissue and development in the insect vector could be found among the animal strains and 2 human strains of *T. cruzi.* All the strains appear to be 1 species differing only in virulence. Virulence as evidenced by infectivity and death rates in mice showed all the animal isolates to be less virulent than *T. cruzi* Corpus Christi strain. Several of the isolates produced infections similar to those observed with *T. cruzi* Brasil, a strain which has lost its virulence in the laboratory. One opossum strain did not infect mice.

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